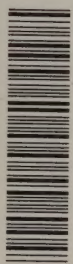




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BACTERIOLOGY

A TEXT-BOOK OF MICROORGANISMS

BY

FRED WILBUR TANNER

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University of Illinois*

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Ever since my life as a man, I do not think I have ever spoken with a student without saying to him, "Work perseveringly; work can be made into a pleasure, and alone is profitable to man, to his city, to his country."

—PASTEUR.

PREFATORY STATEMENT

“A man who writes a book thinks himself wiser or wittier than the rest of mankind; he supposes that he can instruct or amuse them, and the publick to whom he appeals, must, after all, be the judges of his pretentions.”—*Samuel Johnson*.

THIS book has been planned for those who are studying microbiology for the first time. The author is more interested in the opinions of students with regard to the book than in the opinions of those who have long since lost their active contact with beginners, even though they may have positions and titles which imply such contact. If there are students anywhere who desire to offer suggestions, the author would welcome them.

You are about to study one of the most interesting of the biological sciences. There are few courses in the curriculum which can be made more interesting or more useful in everyday life. An opportunity will be offered to study many phenomena about which one hears almost daily. What is the Schick test? Why do we pasteurize milk? Why are certain times and temperatures used in pasteurization? What is certified milk? Why are the fermented milks of value? What is “mother of vinegar”? Is terminal disinfection (fumigation) of real value in the prevention of disease dissemination? What is ptomaine poisoning?—and numerous other questions. A course in microbiology should not be a popular superficial discussion of only the applications of microorganisms. It should be considered as one in a biological science—the study of a group of living organisms subject to all of the fundamental laws to which other groups are subject. There are no sound reasons why we should allow lines of demarcation, established for administrative purposes in our colleges and universities, to separate and divide our knowledge of living organisms. Much of the information which is brought to a course in microbiology from such courses as plant physiology, zoology, human physiology, etc., is just as applicable to the microorganisms as to those forms of life for which it was first learned. The author has never taught introductory bacteriology with the idea that it was an elementary subject. When that is done, too many of those general statements which are only partly correct are introduced. With no other organisms can the great basic phenomena like respiration, fermentation, digestion, etc., be better studied. We

who are teaching must not underestimate the intelligence and abilities of our students by superficial discussions and lectures.

The content of this book is indicated by the title, "Bacteriology—A Text Book of Microorganisms." It is the result of nearly sixteen years' active association with students who are beginning the study of microbiology. The development of bacteriology during the last sixty years has given a wealth of material from which to draw for teaching. There is no reason why a course in microbiology needs to be uninteresting or useless to the student in later life. The great problem of an author in the field to-day is to determine what not to include. The book is intended to be an introduction to the science. Therefore, every attempt has been made to stress the non-pathogenic microorganisms as well as the pathogenic. The author decries the too prevalent custom of taking the student too deeply into pathogenic bacteriology in the first course. Such a course is quite liable to leave an imperfect conception of the field. There is a constant tendency to over-emphasize the disease-producing microorganisms, and to slight classification of bacteria, cytology of the bacterial cell, etc. Might not the present unsatisfactory condition of the classification question, for instance, be in part due to the reticence of teachers to discuss such subjects? Might it not be possible that our literature would have been less cluttered with the names of new species if the problems of nomenclature and the seriousness of reporting new species had been discussed?

Since the page carrying the preface or foreword may be looked upon as an editorial page through which an author may speak directly, to those who use a book, of subjects not considered a part of the subject matter discussed in the context, I wish to call the attention of students to several defects in training which I have observed; first, the reluctance to take courses with laboratory work. One must decide whether one wishes to be a "swivel-chair" scientist or one whose information is well founded on observations made in the laboratory. Roger Bacon, about 1290, emphasized the need for experimentation. Second, the need for the study of the modern languages, especially French and German. Those of us who are concerned with the direction of graduate students are impressed with the large number who come to graduate schools and colleges without such training. Time must be taken from graduate work to make up deficiencies which could have been worked off in undergraduate courses. If the argument that a knowledge of French and German is a necessary tool in our work is not convincing, I may mention the cultural aspects of such knowledge. I feel that the two matters mentioned above are worthy of consideration.

Finally, the author desires to acknowledge the assistance of others.

No man in science lives unto himself. He is constantly acquiring ideas and information from those with whom he works, from his students and, of course, from the literature. I acknowledge helpful suggestions from my colleagues, Dr. G. I. Wallace, and Professor S. A. Koser. Grateful acknowledgment is made to Gustav Fischer of Jena, Germany, for permission to use certain of the illustrations in the chapter on Molds, to Dr. J. J. Schmidt of Hartenholm bei Kaltenkirchen, Germany, for the preparation of photomicrographs in the chapter on Morphology, to various jobbers of scientific apparatus for permission to use illustrations and, finally, to my publishers, John Wiley & Sons, Inc., for their generous cooperation in various ways.

F. W. TANNER.

May, 1928.



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BACTERIOLOGY

A TEXT-BOOK OF MICROORGANISMS

CHAPTER I

HISTORY AND DEVELOPMENT OF EARLY THEORIES

A complete historical discussion of the development of microbiology will not be attempted. Such an attempt would involve the consideration of a great mass of material and the presentation of both sides of long scientific controversies. Consequently, this part of the book is concerned with a number of topics, arbitrarily selected, with which the proper historical setting may be given. The historical foundations of a science are often regarded as monotonous by the student who is studying it for the first time. The situation is quite different, fortunately, in some of the biological sciences, microbiology in particular. In the development of bacteriology, or microbiology, the early biologists were concerned with many fundamental facts and laws, the discussion of which will always be interesting to one who considers Nature and the orderly manner in which she does things.

Undoubtedly the real origin of the science of microbiology or bacteriology is lost in history. As with many other ideas, the science probably developed very slowly as information and data accumulated from scientific observation. One would quickly postulate a sort of nebulous period during which would appear indefinite statements about the possible existence of very minute organisms. These statements would lack experimental proof, at first, but reports of investigations would slowly appear substantiating some of the early prophecies. Such was the case in bacteriology. Some of the early statements lacked absolutely all experimental foundation; still they were surprisingly accurate representations of facts which are well established to-day. They may be found in numerous books dealing with the history of science and medicine; a more fascinating adventure in reading cannot be found. At the end of this chapter is given a short bibliography of the history of medicine and science.

Following this early period was a so-called transitional period during which a few observations gave support to the idea that microscopic organisms might be responsible for many phenomena in nature. Certain observations were made that seemed to suggest the existence of very small organisms, and now and then a bit of experimental evidence was found to support these hypotheses. There was thus a slow change of theory into fact. An example of those early writers who seemed to sense the existence of microscopic organisms was Kircher (or Kircherus). He made his own microscope which, though crude as compared with modern instruments, enabled him to see what he called "worms" in much of the material which he examined. His observations and reports were, perhaps, of little significance beyond showing that microscopic organisms probably existed and stimulating others to take up their study. Like many of the early microscopists he paid little attention to pure cultures but worked with indefinite mixtures of all types of microorganisms.

Generally speaking Anthony Leeuwenhoek (1632-1723), a Dutch officer and later a lens grinder, is considered to have been the first investigator in the field of bacteriology to leave substantial records of his observations. In many respects he may be regarded as the founder of the other biological sciences also, for by his careful observations and technic, even with what we would consider impossible apparatus, he presented through numerous published reports many facts about the bacteria. Leeuwenhoek¹ is given credit by medical historians for a number of fundamental discoveries. He was the first to describe the spermatozoa, the red corpuscles, the protozoa, etc.; and first to find microorganisms in the mouth and on the teeth. Leeuwenhoek's observations are of greater historical interest than of value in themselves. He apparently did not appreciate the necessity of pure cultures; at least, he did not have them, for the illustrations which he published show mixtures of microorganisms. Probably his instruments did not allow him to study the detailed structure of the microorganisms which he reported. However, he is looked upon by many as the founder of microbiology and microscopy. He made careful record of his observations and published papers in the Proceedings of the Royal Society of London. Besides these journal articles he left a number of books all of which show him to have been a discriminating observer.

The Theory of Spontaneous Generation (Biogenesis vs. Abiogenesis).—The theory of spontaneous generation was a product of inaccurately interpreted experiments. The early scientists who interpreted

¹ Harris, D. F. Anthony von Leeuwenhoek, The First Bacteriologist. The Scientific Monthly: 12 (1921) 149-160.

their data to prove the possibility of spontaneous generation were undoubtedly conscientious in their efforts. Their conclusions were wrong because their premises were wrong. It required almost a century of experiment and argument to disprove forever the probability of spontaneous generation. The final overthrow of this theory was accomplished when Pasteur convincingly showed that fermentation was a biological phenomenon and not a mechanical one. The theory of abiogenesis developed slowly. Strange as it may seem, the early investigators who founded the theory arrived at it through attempts to preserve foods or find the real cause of fermentation. It was indeed an important topic for discussion and a fundamental one as well, the solution of which was necessary before other questions could be solved. The writings of all peoples have contained statements supporting the theory. The ancient Greeks believed that life sprang up spontaneously, for did not flies and frogs come from mud? Anaximander stated that animals developed from moisture. These observers lacked the discriminating minds of some present-day workers who appreciate the necessity of having controls in all experiments. Little or nothing was known at that time about reproduction in insects and animals. Very well-known names appear in reviews of the various experiments on this question.

Needham (1728) was perhaps the first investigator to offer experimental data to support the theory of spontaneous generation. He boiled meat and meat juice in corked flasks and stored them for further observation. When these materials spoiled he believed that life had developed spontaneously. He believed that boiling had destroyed all of the "eggs" in the meat juice. Needham, of course, was wrong in this assumption; the boiling had not done this. Here we have a

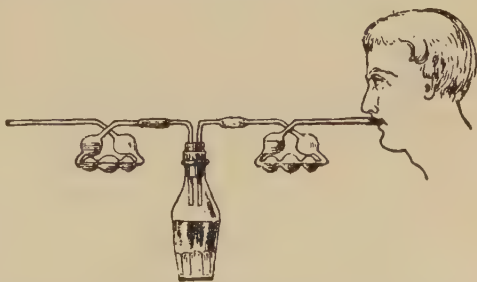


FIG. 1.—Apparatus Used by Schultz in 1836 to Show that Microorganisms were Necessary for Decomposition of Organic Matter.

good example of some possible sources of error in drawing conclusions from experimental work. All of the variable factors must be controlled and understood. Needham ignored some factors which were very important.

Spallanzani, one of the most interesting and reliable workers of these early days, took issue with Needham. Spallanzani stated that the

contents of Needham's bottles spoiled because he had allowed air which had not been exposed to fire (sterilized), to enter. On the other hand, Spallanzani's opponents claimed that in heating the air

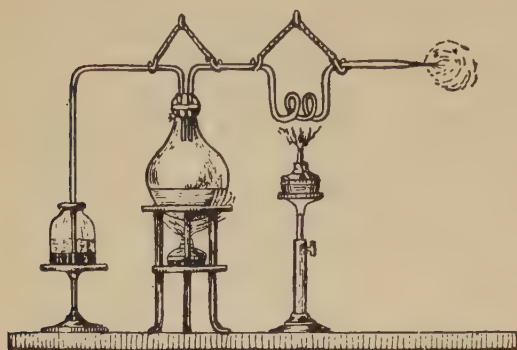


FIG. 2.—Schwann's Apparatus for Disproving the Theory of Spontaneous Generation. (After Loeffler.)

he made it unsuitable for the growth of micro-organisms; they said that his conclusions were also influenced by questionable experiments. Spallanzani answered these arguments by showing that growth resulted when air which was sterilized by other methods was admitted. Similar experiments were also reported by Schultz, who made use of an experi-

ment much like Schwann's; a solution capable of fermentation with yeast did not ferment after thorough heating or exposure to air which had been thoroughly heated. Schwann stated that fermentation was arrested by any influence, such as heat, potassium arsenate, etc., which killed the fungi. On account of this statement, some have said that Schwann was the founder of the science of disinfection. Schröder and Dusch showed that "something" was removed from the air by heating that would permit the growth of micro-organisms. They could not decide whether this agent was itself

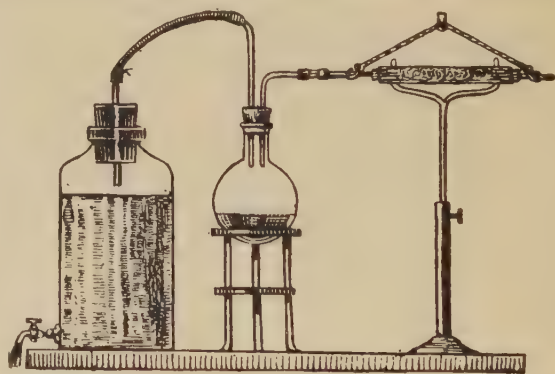


FIG. 3.—Apparatus of Schröder and Dusch for work on Spontaneous Generation.

a living organism or a chemical substance modified by heat. Lamarck, the renowned biologist, believed that the simple organisms developed by spontaneous generation and that there was a progressive increase in complexity from the simplest forms up to man. Very little progress was made against this theory until

the microscope had been perfected to the point where it was no longer a curiosity but a necessary instrument for the study of biology. With the introduction of the microscope, started the rapid downfall of the theory of abiogenesis. In 1668, Redi proved that maggots did not develop in meat from which flies were kept by means of screens.

Pasteur, by means of his convincing experimental data and arguments, finally dealt the theory of spontaneous generation a death-blow. He introduced a fermentable liquid into a flask after which he drew out the neck into a capillary in the shape of a letter S. The flask was then boiled and allowed to cool. It remained sterile for some time but growth appeared in two days after the neck had been broken off. This showed that Pasteur had sterilized the liquid and that it fermented when microorganisms were admitted. As Duclaux has said in his book on

Pasteur, those who argued in favor of this theory, propounded their theory and then attempted to prove it. Pasteur's work was stimulated by an offer by the Academy of Sciences in 1860 for "an attempt by means of suitable experiment to throw new light on the question of spontaneous generation." Pouchet presented a paper before the Academy

of Sciences to show that animals and plants could develop in media in which there were no germs or organic bodies. Pouchet had support from other investigators, which made Pasteur more alert in presenting his side of the question. We have no better illustration of the opposition to many of his discoveries which Pasteur had to meet during the early days of his scientific work.

In leaving this interesting subject of spontaneous generation (abiogenesis), we may well consider it from the strict biological viewpoint. What sort of a nature would we have if life did originate spontaneously? Could Nature do her wonderful work with such a haphazard method for the origin of living beings? A consideration of such fundamental questions soon leads us into fields where we have few scientific data.

Having accepted the doctrine that spontaneous generation does not occur at the present time, we must admit that biologists have some difficulty in explaining the primal origin of life. When stating that life generates life (one cell develops from another) biologists still have to admit that at some early age there was a transformation of inanimate matter into animate matter or that spontaneous generation did then



FIG. 4.—Flask of a Type Used by Hoffmann and Pasteur in Their Work on Fermentation.

take place, after which life was continued by cell division. We, however, are not in position to tell what took place at these early times. We find it difficult to refrain from interpreting these phenomena in the light of present-day experiences. Such a state of affairs is liable to introduce errors into our arguments and opinions.

There will probably always be those who believe in spontaneous generation. It is still believed by many that horse hairs develop into snakes. Some still believe that snakes and frogs are rained from the heavens. In many cases those who express such belief have never seen the phenomena on which they speak with such authority, but must quote the statements of others.

The Germ Theory of Fermentation.—Some of the earliest scientific work in bacteriology was concerned with the establishment of the germ theory of fermentation. In establishing this theory, several other problems were also settled,—the proof for the germ theory of disease and refutation of the theory of spontaneous generation. Fermentation had been carried out for centuries and man knew how to propagate the fermentation organisms although he did not know just what was going on. Solution of the process was not attempted until about 1697 when Stahl² founded the hypothesis which Liebig later attempted vainly to prove. Stahl proposed that fermentation resulted from, or was, a shattering of molecules due to some force set up in the molecules or in the medium about them. This was transferred to other molecules. Finally parts of the molecules were forced out of the liquid. There seems to have been no explanation of just how the energy transfer which takes place, started. Such a conception seems out of tune with the general manner in which phenomena in nature are carried on. Fabroni seems to have been one of the first investigators to believe that some form of life was concerned when sugar solutions were fermented. He noticed that the deposits in many fermenting solutions were composed of cells possessing an albuminoid structure. This seems to be one of the first facts discovered about that interesting and wonderful yeast cell. Thenard³ did not accept Fabroni's report.

In 1810 Appert⁴ reported studies which were directly concerned with this question. In response to an offer of 20,000 francs by the French government for a method of preserving foods, he founded the modern

² Stahl, G. E. *Zymotechnia fundamentalis*. 1734.

³ Thenard, L. J. *Mémoire sur la fermentation vineuse*. *Ann. Chim. Phys.*, **46** (1803), 294-320.

⁴ Appert, N. *The Book for All Households or The Art of Preserving Animal and Vegetable Substances for Many Years*; translated by K. G. Bitting, M. S. Glass Container Association of America. Chicago, Ill., August, 1920.

canning industry by preserving various foods in sealed containers which were afterward subjected to the influence of boiling water. Appert did not attempt an explanation of the factors which made the food keep but others who had given more attention to the phenomenon of fermentation did. One of the first among these was Gay-Lussac,⁵ who stated that air was necessary for fermentation and spoilage of food packed by Appert's method. As long as air was excluded no decomposition was possible. He opened a bottle of grape juice in such a manner that part of it was collected over mercury away from air and the other part in the presence of air. The latter portion fermented and it was, perhaps, logical that the air should be selected as the determining factor. At that time living agents which could cause fermentation were unknown.

Berzelius⁵ at Upsala gave much support to the *mechanistic* conception of fermentation. His position in the scientific world in 1839 added great weight to any side of a controversy with which he aligned himself. With much scorn he pointed out what he considered to be errors in the experiments of those who believed that fermentation was a biological phenomenon. He stated that a certain force was functioning by which fermentation was initiated and kept going. The statements of Berzelius were generally accepted by scientists of his day and many were attracted to his banner. After Fabroni, Cagniard-Latour⁶ stated that fermentation was caused by yeast. This conception received support in observations by Schwann. He showed that heating air and boiling fermenting liquids prevented further decomposition. Schwann's⁷ experiments led him to believe that fermentation and putrefaction were biological processes. Kützing⁸ also proposed that yeast cells were necessary in fermentation. He believed that yeast was an organized form of life.

Attention may now be given to that interesting controversy over the real nature and cause of fermentation which took place between Pasteur, on the one hand, and Liebig and his followers on the other. Liebig⁹

⁵ Gay-Lussac, F. J. Extrait d'un Mémoire sur la Fermentation. Ann. Chim. Phys., **76** (1810), 245-259.

⁵ Berzelius, J. Berzelius' Jahresbericht **18** (1893), 400-403.

⁶ Cagniard-Latour. Mémoire sur Fermentation Vineuse. Ann. Chim. Phys. **68** (1838), 206-222.

⁷ Schwann, T. Vorläufige Mitteilung betreffend Versuche über die Weingärung und Faulins. Ann. Physik. **41** (1837), 184-193.

⁸ Kützing, F. Mikroskopische Untersuchungen über die Hefe und Essigmutter nebst mehreren anderen dazugehörigen vegetabilischen Gebilden. Jour. pr. Chem. **11** (1837), 385-409.

⁹ Liebig, J. Ueber die Erscheinungen der Gärung und die Quelle der Muskelkraft. Annalen **153** (1870), 1-47; 137-228.

argued the older contention of Berzelius that fermentation was a mechanical process as opposed to a biological. Berzelius had believed that the yeast had acted in fermentation by virtue of a catalytic force which he had hypothecated to exist. Liebig did not admit this and did not believe that yeast cells were necessary in fermentation. He believed that air in contact with saccharine materials such as plant juices caused the formation of a ferment, a body probably quite different from the later agent indicated by that term. This agent was believed to undergo rapid change of the nature of putrefaction or decay. This ability to be in a constant state of change was imparted to the sugar molecules. Liebig's theory may, then, be regarded as a mechanical explanation rather than a biological explanation.

Pasteur¹⁰ stated that this was not a tenable explanation of the forces which caused fermentation; he said fermentation was a biological process brought about by yeast. He published several papers about 1860, all of which dealt with the relation of microorganisms to fermentation.¹¹ The world of science was in a happy condition and the value of science was being appreciated more and more. Chemistry had made rapid progress and new compounds were being made and discovered every day. Pasteur noticed such compounds as amyl alcohol among the products of fermentation.¹² He found it difficult to explain their presence according to Liebig's theory. The structure of amyl alcohol was too different from that of sugar to consider it an integral part of the sugar molecule. We need not burden ourselves with the details of these controversies. The necessity of living cells in fermentation is now accepted.

After the germ theory of fermentation had been established further studies were carried out to determine the details of the phenomenon. Traube¹³ made one of the first contributions to the subject by founding the modern enzyme theory. He tried to reconcile the opposing views of Pasteur and Liebig. It became apparent that there were definite agents, now known as enzymes, in cells which could cause fermentation. This was finally confirmed by Buchner's work on zymase in 1897. In support of Traube's papers appeared those of Berthelot¹⁴ who isolated the

¹⁰ Pasteur, L. *Mémoire sur la Fermentation Appelée Lactique*. *Comp. Rend.* **45** (1857), 913-916.

¹¹ Pasteur, L. *Mémoire sur la Fermentation Alcoolique*. *Ann. Chim. Phys.* **58** (1860), 323-426.

¹² Pasteur, L. *Mémoire sur la Fermentation Lactique*. *Ann. Chim. Phys.* **52** (1858), 404.

¹³ Traube, M. *Theorie der Fermentwirkungen* Dummlers Verlagsbuch. 1858, 119.

¹⁴ Berthelot, M. *Sur la Fermentation Alcoolique*. *Comp. Rend.* **44** (1857),

enzyme invertase. The work of Traube stimulated a long line of investigation. A German worker, Lüdersdorf,¹⁵ had tried to find an explanation by testing the fermenting ability of ground yeast cells. No fermentation resulted. After this a number of investigators, Mayer¹⁶ Nageli, and others, added to the information of the day.

The question of the presence of enzymes in yeast capable of inducing the alcoholic fermentation was finally settled by Buchner¹⁷ when he produced a yeast juice which in the sterile condition would cause the fermentation of saccharine materials. Buchner's work might indicate that Liebig had some basis for his contention in his controversy with Pasteur. Indeed some chemists have stated that Liebig was partially correct; such a statement is not in accord with the facts. Liebig stated that fermentation was a mechanical phenomenon and that living agents were not concerned. According to Buchner's work, living yeast cells must form the zymase even though it may function away from the cells once it has been prepared from them. No one has been able to synthesize an enzyme.

In modern times important investigations are being reported. These, of course, will need the perspective of time to show their merits. Chief among these are the researches of Neuberg and his collaborators, who have so greatly elucidated the chemistry of alcoholic fermentation.

EARLY WORK IN MEDICAL BACTERIOLOGY

In the earliest historical records there are frequent allusions to the possible relation between microscopic organisms and certain diseases. It remained for investigators of the nineteenth century to supply the evidence for the knowledge which we now possess. There were few reasonable explanations for disease and one does not wonder that invisible microorganisms were suggested as a possible cause. Some writers give credit to Geronimo Fracastorio (1484) for being the first person to explain disease in accordance with present-day ideas. Kircher, as stated before, also seemed to have a clear conception of the relation of microorganisms to disease. Plenciz, an Austrian physician, proposed a germ theory of disease and even proposed a separate organism for each disease, thus suggesting the specificity which we now recognize.

702-706. Sur la Fermentation Glucosique du sucre de canne. *Comp. Rend.* **50** (1860), 980-984.

¹⁵ Lüdersdorf, F. Ueber die Natur der Hefe. *Ann. Physik.* **76** (1846), 408-411.

¹⁶ Mayer, A. *Lehrbuch der Gärungschemie*, 3. Aufgabe. Carl Winters. Univ. Buchh., Heidelberg, 1879. Nageli, C. *Theorie der Gärung*. München, 1879.

¹⁷ Buchner, E. Alkoholishe Gärung ohne Hefezellen. *Berichte* **30** (1897), 117-124.

We may now go immediately to some outstanding pioneer workers who made great contributions to the science, leaving for future study the other investigators of these and earlier days. There may be some difference of opinion over who should be included in such a review, the five or six mentioned below having made sufficiently significant contributions. However, no significance should be attached to the order in which their contributions are discussed as their most active years coincided quite closely.

Robert Koch (1843-1910) : Koch was a German trained in medicine. He started practice as a physician and became interested in the work of Pasteur, since he had begun to doubt the miasmatic theory of disease causation. His earlier work on anthrax and staining procedures brought him into such prominence that he was called to the imperial health office in Berlin. Koch soon brought other workers, all of whom became well known for discoveries in the etiology of disease. Besides the discoveries in this field, Koch also interested himself in work on sterilization, disinfection, media, staining, etc., subjects which had to be studied before progress could be made in the field of applied bacteriology. One of the first things for which we may remember Koch was his introduction into bacteriology of liquefiable solid media. After developing such a medium it became possible to isolate bacteria in pure culture; making the progress of medical bacteriology more rapid. Dilution in liquid media had been used up to this time. With this method results were uncertain. Our textbooks of the present day show the impetus which was given to studies in the causes of disease, for within about seven years after this important advance in technic, the etiology of many of our common diseases was worked out. Some of these were investigated and solved in Koch's own laboratory.

Koch is also known as the discoverer, in 1884, of *Mycobacterium tuberculosis* (*Bacterium tuberculosis*). He described the preparation of tuberculin and believed that it could be used for the prevention of tuberculosis. Other interests and contributions may be tabulated.

1876, First report on the etiology of anthrax.

1878, First report on etiology of wound infections.

1882, Used gelatin as a liquefiable solid medium.

1883, With Cholera Commission in Egypt.

1884, Discovery of *Mycobacterium tuberculosis*.

Louis Pasteur (1822-1895) : This investigator made many of the fundamental discoveries in bacteriology and chemistry. Pasteur's first interest was in chemistry but later his attention was directed to problems in microbiology and biochemistry. His first real contribution to science was on molecular dissymetry in 1848. He found that racemic modifications of tartaric acid could be separated into dextro- and laevo-rotatory acids. He also found that the dextro-rotatory tartaric acid could be fermented by microorganisms and by this experiment he was probably introduced to the study of microorganisms.

Pasteur's next contribution, to which we shall refer, concerned fermentation. He believed that fermentation was a biological phenomenon, induced by living cells (bacteria, yeasts, etc.) in opposition to the views of the great German dictator in chemistry of that day, Justus von Liebig. Pasteur also studied the "diseases" of wine, vinegar and beer, reporting that these abnormal fermentations could be stopped by heating the bottles to between 55° and 60°C., a process which later became known as pasteurization.

The etiology of *pebrine*, a disease of silkworms, was solved by Pasteur through which he, probably, received his inspiration to attack diseases of higher forms of life. The silkworm industry was an important one in certain districts in France. Pasteur and his colleagues spent five or six years in a little laboratory near Alais in intensive research; as a result he discovered the cause and means of prevention of *pebrine*.

Pasteur's solution of the silkworm disease furnished one of the strongest defenses for the germ theory of many diseases. Years of research since then have established it beyond all doubt. Pasteur was reluctant to undertake this investigation because he knew very little about silkworms and had never handled one. Promptings on the part of his old teacher caused him to undertake the study which ended so satisfactorily and which opened the eyes of Pasteur to the whole field of human and animal disease.¹⁸ The entire account has been well described by Valéry-Radot, and also by Duclaux.

Pasteur's success in the solution of the silkworm disease stimulated him to turn his mind to other diseases which were causing great losses in France. Anthrax was receiving much study in various places. Anthrax vaccine was prepared and convincing demonstrations of its value were made. These are well described in the biographies of this great scientist.

In the field of human diseases Pasteur's work forms the solid foundation on which many of our present-day practices rest. He took up the study of anthrax, confirming Koch's work that the organism (*Bacillus anthracis*) could be propagated for a great number of generations under saprophytic conditions to cause the disease when reintroduced into a susceptible animal. When he discovered the value of anthrax vaccine he started a method of combating disease which has been of great value. This discovery, while not accidental in the strict sense, was unexpected. He left his laboratory for a time, and found that cultures of the virus of chicken cholera had become sterile but possessed protective powers for when they were injected into a chicken this chicken was not susceptible to infection from a virus of known virulence. Later on in a public demonstration he showed that he could protect sheep from anthrax by vaccination. The account of this in Pasteur's biographies is stimulating and clearly illustrates Pasteur's method of bringing controversies to a head. In numerous instances, he offered to resort to demonstration to convince others of his discoveries.

To a student in the sciences Pasteur is a wholesome example.¹⁹ He was a

¹⁸ Pasteur, Louis: His Life and Labors. By his Son-in-Law (Translated by Lady Claude Hamilton). Appleton, New York.

¹⁹ Duclaux, E. Pasteur: The History of a Mind. (Translated by E. F. Smith and Florence Hedges.) W. B. Saunders & Co., 1920.

loyal son of France and every discovery which he announced was looked upon as being done for "beloved France." He was profoundly religious and a true gentleman. Despite the fact that he is revered to-day in all lands as one of the greatest benefactors of the human race, and received the highest honors before his death, Pasteur had to defend many of his discoveries in controversies which became very bitter. Many of these heated arguments occurred before the Academy of Sciences where were gathered the most eminent men in France. One can understand the antagonism and criticism that might come from foreign countries, and how Pasteur would feel, but it is more difficult to understand that which came from his colleagues in France.

Elie Metschnikoff (1845-1916): Metschnikoff was born in Russia and educated as a biologist. He made his first contribution to biological literature in 1865. After many years as a biologist Metschnikoff suddenly changed to a pathologist. His early work had slowly led him to report what we know to-day as his theory of phagocytosis. According to this, the white cells in the blood serve as a defense of the organism against intruding bacteria. After establishing this theory he had to spend much time in later life defending it. Metschnikoff worked for a time in a municipal bacteriological laboratory at Odessa. This institute was opened in 1886. He concerned himself with infectious diseases but was soon compelled to take up the defense of this theory of phagocytosis. Working conditions at the Odessa institute soon became so unfavorable that he began to look for a new location where he could carry out his researches in a more peaceful environment. Since Emmerich had attacked his theory of phagocytosis, Metschnikoff visited him at Munich and decided not to locate there. He also went to Paris to visit Pasteur, where he was received courteously by this great man. Metschnikoff inquired whether he might work in an honorary capacity in Pasteur's laboratory. Pasteur's cordial invitation made a great impression on Metschnikoff, but since he was doubtful about research conditions in a large city, he desired to look about a little more. On his way back to Odessa from Paris, he stopped at Berlin to see Robert Koch and showed him some specimens of phagocytosis. Koch received him quite coldly and refused to admit any value in his theory of immunity. This caused Metschnikoff to decide to go to Paris with Pasteur. He arrived there in 1888. Here he found congenial friends and an ideal place for work.

Metschnikoff is also known for his views on some of the causes of premature death. He believed that one of the characteristic symptoms of old age was hardening of the arteries. This, in turn, was believed to be due to autointoxication due to the presence of proteolytic bacteria in the intestines. Metschnikoff suggested that these protein-splitting bacteria be replaced by acid-forming bacteria in order to avoid the formation of toxic products. Thus Metschnikoff proposed another theory which required a great amount of study to elucidate.

Metschnikoff died in 1916, his last years being somewhat unhappy ones. He was depressed by the World War which took away from the Pasteur Institute so many research workers. His grief was intensified as he heard of the death of his young colleagues in the War.

Joseph Lister (1827-1912): Lister is another of those pioneers who lived and worked during what may be called the golden age of bacteriology. Unlike Pasteur, he was trained in medicine. Lister's contributions were very important. As an interne and hospital surgeon, Lister was impressed by the appalling mortality from infections in hospitals, just where such things should not occur. Hospitals in those days were quite different from those of to-day. Clean surgery, spoken of as aseptic surgery, was born in 1867. Lister's noteworthy contributions to bacteriology and medical science were related to wounds and operations. Pasteur had already demonstrated that bacteria were concerned with putrefaction and fermentation. Lister discussed these discoveries in relation to his own ideas as follows:

"Turning now to the question how the atmosphere produces decompositions of organic substances, we find that a flood of light has been thrown upon this most important subject by the philosophic researches of M. Pasteur, who has demonstrated by thoroughly convincing evidence that it is not to its oxygen or to any of its gaseous constituents that the air owes this property, but to minute particles suspended in it which are the germs of various low forms of life, long since revealed by the microscope, and regarded as merely accidental concomitants of putrescence, but now shown by Pasteur to be its essential cause, resolving the complex organic compounds into substances of simpler chemical constitution, just as the yeast plant converts sugar into alcohol and carbonic acid."

Pasteur's work thus suggested to Lister the cause for the high mortality from wounds. He realized that some effort must be made to prevent the access of bacteria to such wounds or to destroy those which have gained entrance. For this purpose, Lister employed phenol (carbolic acid) without knowing that Semmelweis and Lemaire, and perhaps others, had used this compound before.

In order to appreciate fully the significance of Lister's work one must know something of surgery in pre-Listerian times. When he was an interne in the University College Hospital he had opportunity to observe at first hand the infections which followed surgery, child-birth, etc. The surgery of the day was in a pitiable condition. Hospitals of that time (1860) were seething beds of infection. The mortality was terrific and every precaution was taken to check it, to no avail. These attempts had to do mainly with improvements in hospital construction, ventilation, etc. After careful study Lister was convinced that inflammations and the accompanying suppuration were caused by certain bacteria. If they could be eliminated, these terrible conditions would be prevented. As has been stated above, the chemical with which Lister proposed to do this was phenol or carbolic acid; the actual material which he used was known as German creosote. With respect to one case, Lister wrote:

"There is one of my cases at the infirmary which I am sure will interest thee. It is one of compound fracture of the leg with a wound of considerable size and accompanied by great bruising and great effusion of blood into the substance of the limb, causing great swelling. Though hardly expecting

success I tried the application of carbolic acid to the wound to prevent decomposition of the blood and so avoid the fearful mischief of suppuration throughout the limb.

"Well, it is now eight days since the accident, and the patient has been going on exactly as if there were no external wound—that is, as if the fracture were a simple one. His appetite, sleep, etc., good, and the limb daily diminishing in size, while there is no appearance whatever of any matter forming. Thus a most dangerous accident seems to have been entirely deprived of its dangerous element."

War surgery before 1850 was also in a terrible state. Many know of the incident when Paré who, when the Duke of Guise was struck with an arrow which buried its head in his face, put his foot on the subject's face and by sheer force pulled out the arrow. That was surgery in those days. Wounds were expected to fester and become putrid; the surgery of the day was mostly concerned with external injuries, etc. Very few attempts were made to explore the larger cavities of the body or to perform operations on the internal organs. With the introduction of chloroform in 1850 operations increased and consequently deaths from surgical fever also increased. One lay-writer discussed the situation as follows:

"No newspaper would print a faithful description of the surgical wards of the pre-Listerian era. Horrible as the subject is, it is necessary to mention it in order to understand the true proportions of Lord Lister's achievement. What the modern surgeon calls a clean and sweet wound was all but unknown. From any wound, whether surgical or accidental, there was almost invariably a discharge of decomposing matter that frequently led on to gangrene and death. Because such conditions developed only where blood and tissue were exposed to the air, it was assumed that they were due to a contagion in the air; that the patients in a surgical ward poisoned not only each other but also the very rooms in which they lay. It was seriously proposed at one time, that the hospitals of the future should be cheap and temporary buildings, which could be burned down as soon as the inmates had saturated them with poison.

"This was the state of the surgical wards, not a few centuries ago, but as recently as the early 1860s, when Lister first heard of Pasteur's great discovery that putrefaction was not due to contact with the air itself but to minute living organisms somewhat vaguely described as 'germs.'"

Before leaving the work of Lister, Semmelweis (1818–1865) who studied the causes of puerperal fever should, at least, be mentioned. He applied disinfectants to the wound after childbirth and secured satisfactory results. He preceded Lister by about twenty years.

Edward Jenner (1749–1823): Jenner, another pioneer worker in preventive medicine, secured part of his training under the great surgeon, John Hunter. He had to travel a path of resistance which was just as severe as that traveled

by other investigators who made significant contributions to science. Jenner was repeatedly threatened with death if he dared to practice the discoveries for which he is remembered. He heard of the tradition that milkmaids who contracted cow-pox from the animals which they milked, never had small-pox. He decided to investigate this rumor and after convincing himself of its soundness, attempted the artificial inoculation of a boy named James Phipp. This boy was later inoculated with active small-pox virus but did not take the disease. Jenner continued his work and published a number of contributions to the subject of vaccination. His ideas were soon taken up by many European students who secured the same satisfactory results. They were not, however, widely accepted by all classes of people. In his day, just as at the present time, some of those regarded as most intelligent fought the practice of vaccination.

EARLY WORK IN AGRICULTURAL BACTERIOLOGY

Certain practices in agriculture were followed for centuries before it was dreamed that hosts of minute organisms were thus being favored to bring about desirable changes in the soil. Conn has said that the intelligent farmer of to-day is one who controls bacterial life. Agricultural bacteriology has been developed along many different lines, and it would be difficult and unnecessary to discuss them in detail.

Soil fertility has been of interest since the earliest times. We are told ^{19a} that Columella mentioned in his writings the practice of Roman farmers, of plowing under lupines to enrich the soil. Other writers also made statements which show that they appreciated the value of crop rotation and other agricultural practices for which they had no scientific explanation.

That certain crops, such as the legumes, could increase the nitrogenous content of the soil first received definite study at the hands of Schultz-Lupitz.²⁰ Fifteen crops of lupines were raised on a sandy soil with a constant yield. Cereals grown on this same ground yielded larger crops than when grown on ground which had not supported the growth of lupines. Chemical analysis showed that the nitrogen content of this soil had been increased 0.06 per cent. Explanations of this increase in the content of nitrogen were sought which, after careful investigation, led to the discovery of symbiotic and non-symbiotic nitrogen fixation.

That certain plants, the legumes, could obtain atmospheric nitrogen by means of organisms living in nodules on their roots was established by Hellriegel and Wilfarth.²¹ A more definite statement was made by

^{19a} Marshall's "Microbiology," 1911, 273.

²⁰ Schultz-Lupitz. Landw. Jahrb. **10** (1881), 777.

²¹ Hellriegel and Wilfarth, Tagblatt d. Naturforscher Versamml. z. Berlin.

Hellriegel²² in 1888. The nodules on leguminous plants had not gone unnoticed. Malpighi in 1687 had noticed them but regarded them as pathological conditions.

Beijerinck²³ in 1888 reported the organism which fixed nitrogen symbiotically with legumes to be *Rhizobium leguminosarum* (*Bacillus radicicola*). The fixation of nitrogen without the intervention of symbiosis was also discovered. Winogradski²⁴ isolated the organism *Clostridium pasteurianum*. Other organisms have since been found which will fix nitrogen non-symbiotically.

The subject of nitrification must also be mentioned in this connection. In 1878 Warington²⁵ showed that certain antiseptics such as chloroform and carbon bisulphide would prevent nitrification. He also stated that two steps were involved. However, it remained for Winogradski²⁶ in 1890 to isolate the organisms which are concerned with this oxidation.

Burrill established the relation of bacteria to plant diseases when, in 1878, he stated that *Erwinia amylovora* was the cause of pear blight. Healthy trees when inoculated with the material from diseased trees, were blighted. This work founded what we know to-day as plant pathology and was one of the first real attempts to connect bacteria with plant diseases. Since that time progress has been rapid, and this phase of the science has become an important one.

DEVELOPMENT OF THE LENS AND MICROSCOPE

Before rapid progress could be made in microbiology, microscopes with which to study microorganisms had to be developed. The real originator of the microscope may never be known. Before microscopes could be made, however, the lens had to be discovered. Lenses are the most important parts of the microscope. The science of optics developed slowly even after some of its basic principles had been discovered. Some of them were probably accidental discoveries. The date for the beginning of the science of optics depends somewhat on the interpretation which is placed on some of the early reports. In the Encyclopedia

²² Hellriegel and Wilfarth. See Lafar's Handbuch tech. Mykologie, 1904-06, **3**, 31.

²³ Beijerinck, Bot. Zeit. **46** (1888), 725.

²⁴ Winogradski, S. (1895). Recherches sur l'assimilation de l'Azote libre de l'Atmosphere par les Microbes. Archives des Sci., Biol. **3**, 297-352.

²⁵ Warington, R. "On Nitrification." Jour. Chem. Soc., **33**, Pt. 1, 44-51. Papers also published in the volumes for several succeeding years.

²⁶ Winogradsky, S. Recherches sur les Organismes de la Nitrification. Ann. Past. Inst. **4** (1890), 213-231; 257-275; 760-771.

Britannica it is stated that a convex lens of rock crystal was found among the ruins of the palace of Nimrud. Seneca mentioned the increased brilliancy of the colors of fruit when they were viewed through or were placed in globes of water.

Singer, whose interesting paper has been consulted, stated that lenses may be traced back to the thirteenth century and that an earlier origin is not improbable. He believed that the gem cutters of antiquity must have used some apparatus for magnifying objects else they could scarcely have done such fine cutting. Pliny stated that burning glasses were used by physicians as cauteries. Carpenter believed that the suggestion of the early existence of lenses on the grounds that they were necessary for fine gem cutting was unnecessary for some persons are endowed with greater visual powers than others. He also mentioned the fact that no Greek or Roman writer mentioned their use. Some of the early Greek physicians stated that myopia was incurable; such statements appeared even toward the end of the thirteenth century. If the rock crystal mentioned above was used as a lens, it would place an earlier date for the beginning of the science of optics.

Roger Bacon (1214-1294): Bacon was a Franciscan Monk who published several works of great interest to students of science. He is given credit for developing the first convergent lens and, therefore, the first simple microscope. Bacon is probably the founder of the science of optics. He emphasized what philosophers of his day neglected, that it was only by experiment that real progress was made in science.

Although Bacon appreciated the necessity of experimentation it was not until some centuries later when such men as Newton, Harvey, Galileo and others began to work that the experimental method was finally used for seeking after truth. Previous to this time scientists were content with discussing the writings of Aristotle, and other early writers. Anyone who disagreed with these "authorities" was regarded as revolutionary and not to be trusted. Such was the situation with many early scientists such as Harvey, Jenner and even with Pasteur.

About the year 1300 spectacles were introduced by Salvino d'Amato degli Armati of Florence and Allesandro de Spina of Pisa. Some historians give Bacon credit for the introduction of spectacles. According to Carpenter, a manuscript from Florence dated 1299 has this statement:

"I find myself so pressed by age that I can neither read nor write without those glasses they call spectacles, lately invented, to the great advantage of poor old men when their sight grows weak."

In the sixteenth century the lens was adapted to the study of nature. We shall not attempt a complete review of those who were concerned

with the development of the microscope but will single out several of the more important workers.

Gage in his "The Microscope" called attention to the wonderful engineering feats of the Ancients without instruments which are regarded as so necessary by engineers of to-day. There were no levels and transits with which to lay out the famous aqueducts, pyramids, etc.

Some of the real early work was done with the simple microscope, a natural development of the lens. The early instruments of this type consisted of a tube of opaque material with the lens in one end and a flat glass plate on which the object was placed in the other. Such an instrument used by Kircher is shown in Fig. 8.

Galileo (1564-1642): There is much evidence to favor Galileo as the discoverer of the compound microscope in 1610. It has been stated that he developed the microscope from the telescope. Galileo announced that his "occhiale" made flies look like hens. He is not often thought of in this connection, but the evidence presented by Carpenter is convincing. Galileo apparently did not follow up his discovery with reports of its revelations, as did Leeuwenhoek and others, and therefore does not receive the credit due him as one of the discoverers of the microscope. It has been recently stated that this great Italian's name is Galilei and not Galileo.

Borel (1620-1671): Borel was one of the first to use the microscope as an aid in the study of diseases. He probably had little to do with the development of the microscope, but used it in his profession. Singer believed that Borel probably discovered the corpuscles in the blood as early as 1653. Borel, also, published the first book on microscopy as such.

Hooke (1635-1703): The work of Hooke in cytology gives him a prominent place in historical discussions of microscopy. In 1665, he published his "Micrographia" showing his compound microscope. Hooke was well trained but cannot be regarded as a great biologist. He first described what we know to-day as cells.

Faber: Giovanni Faber (1625) introduced the name microscope.

Zacharias²⁷ (miscalled Jansen according to Singer): A son of a spectacle-maker discovered the principles of the telescope by using an instrument consisting of two lenses in a tube. He was, then, one of the originators of the compound microscope.

Kircher (1601-1680): Athanasius Kircher is another pioneer in microscopy who is given little attention by bacteriologists. He made a compound micro-

²⁷ Singer stated that the use of the name Jansen for this investigator is due to a misunderstanding; Zacharias was, indeed, the son of John the spectacle-maker but Jansen was not in this case a surname. This man and his son are spoken of by others as Hans Jansen and his son Zacharias.

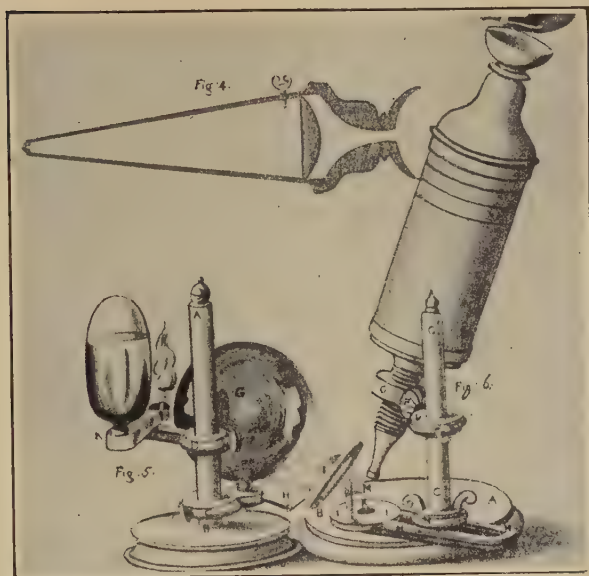


FIG. 5.—Hooke's Compound Microscope. (After Singer, 1914.)



FIG. 6.—Zaccharias Jansen, from Borel's "De Vero Telescopio Inventore." (After Singer, 1914).



FIG. 7.—Athanasius Kircherus. (After Singer, 1914.)

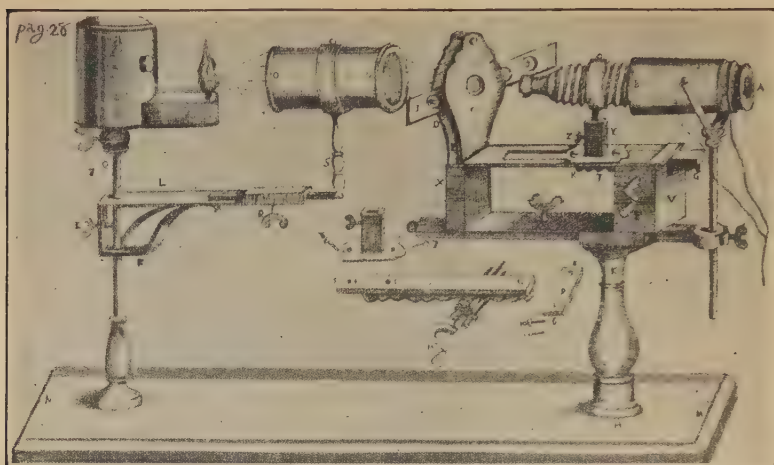


FIG. 8.—The Compound Microscope of Athanasius Kircherus Adapted with Coarse and Fine Adjustments and a Substage Condenser. (After Singer, 1914.)

scope which was used for the collection of data published in a number of papers.

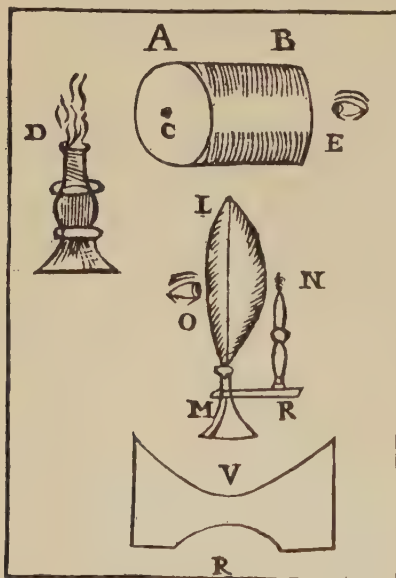


FIG. 9.—Simple Microscope of Athanasius Kircherus. (After Singer.)

The object to be examined was placed on the glass plate at *C*; it was illuminated by the candle *D*; the lens was at the opposite end *E* of the tube *AB*.

instruments which he devised and used. Nearly all of Leeuwenhoek's lenses

He made quite intensive studies in the microscopic world and stated that air and water teemed with a myriad of small organisms. He reported the presence of "worms" in many materials such as decaying meat, vinegar, etc. Kircher did not publish such detailed reports as did Leeuwenhoek and is consequently less known.

Leeuwenhoek: We have already discussed some of the work of Leeuwenhoek. In 1673 this pioneer in microscopy began to send his papers to the Royal Society of London. In contrast with Hooke and others Leeuwenhoek was not a university trained scientist. Locy has given a fine description of this enthusiastic pioneer in microscopy. It seems that microscopy was somewhat of a hobby with Leeuwenhoek since he held a minor court position for a livelihood. In this place we may be more directly concerned with the

were simple ones ground by himself. Loezy stated that he possessed not less than 247 complete microscopes and 419 lenses which were used in his investigations. One of his microscopes is in the possession of the University of Utrecht. Leeuwenhoek was not a systematic investigator, and consequently his interests were quite plastic.

PRESENT-DAY SITUATION

The situation to-day is quite different from that of the times to which we have just referred. There has been great specialization and division of the science, as mentioned below, into a number of sections.

General Bacteriology.—

This phase of the science deals with bacteria and related microorganisms, as living beings, objects for study in the same manner as plants and other animals. It should be studied before venturing into any of the divisions of applied bacteriology. Here the student will consider the bacteria as living organisms, and lay a broad solid foundation. Later on in this book some of the useful applications of bacteria to industrial life are discussed.

Dairy Bacteriology.—This division of the science is concerned with the relation of bacteria to milk and other dairy products. It was one of the earliest applications of the science to practical work. Conn at Wesleyan University, Middletown, Connecticut, and Russell at the University of Wisconsin were the first dairy bacteriologists in America. The numerous publications of these investigators soon stimulated others to enter the field. Almost every Agricultural Experiment Station in America has its own dairy bacteriologist; besides these there are numerous bacteriologists working with private dairy plants, and state boards of health. The American Public Health Association has published several editions of *Standard Methods for the Examination of Milk*.

Sanitary Bacteriology.—This division of bacteriology, although it may include various applications of bacteriology to conservation of

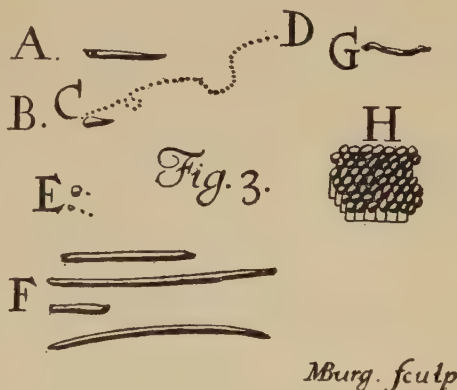


FIG. 10.—Bacteria as Reported by Leeuwenhoek, 1683. (After Loeffler.)

E, Cocci; A, F, and G, rod-shaped organisms; H, Sarcina; C, Flagellated Organisms.

health, usually refers to water and sewage. As the density of population increased in certain sections, notably about London and Boston, it became necessary to treat the water and sewage. Research institutions, also, had to be founded where information could be collected on water and sewage treatment. Among the several institutions of this nature which could be mentioned are the Lawrence Experiment Station at Lawrence, Mass., the Illinois State Water Survey at Urbana, Illinois, and the research laboratories of the Metropolitan Water Board, at London, England. Every intelligent citizen should be interested in the source of water and the methods of sewage disposal for the community in which he lives.

In America the American Public Health Association has done much to stimulate the study of methods for the examination of water and sewage. Through some of its committees, this association has published "Standard Methods for the Examination of Water and Sewage," a revision of which appears about every five years.

Soil Bacteriology.—Soil bacteriology or soil biology has become an important division of the general field of microbiology. This phase of bacteriology is concerned with the activities of microorganisms in the soil. Bacteria play a great role in soil fertility; this necessitates continuous study for new facts and methods of working. There are many centers where soil bacteriology may be studied. Almost all of the Agricultural Experiment Stations have departments or divisions of soil biology.

Industrial Bacteriology.—Microorganisms are taking a more important place in the industries than heretofore. The World War did much to stimulate the use of microorganisms for the preparation of chemicals which before were prepared by chemical methods. The use of microorganisms in this respect is not new. Bacteria and yeasts have been used for ages in the preparation of vinegar. Yeasts have been used for centuries for the manufacture of fermented drinks and later for the production of ethyl alcohol. More recently they have been used for the manufacture of glycerol. Bacteria are used to-day for the preparation of butanol and acetone. Molds are used in the amylo process for the manufacture of ethyl alcohol.

The future of this phase of microbiology is very encouraging. Each yeast cell, for instance, may be considered as a miniature factory in which chemical changes are brought about. Some of them would require a great amount of apparatus and materials to be induced by ordinary methods in the laboratory. Soil biologists have drawn the analogy between the roots of legumes wherein bacteria may fix nitrogen and the great chemical institutions wherein nitrogen is fixed chemically.

In both instances nitrogen is taken from the atmosphere and fixed on the earth.

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CHAPTER II

SYSTEMATIC RELATIONSHIPS OF MICROORGANISMS

Scientists working with living organisms have found it necessary to make systematic classifications. Living things have been divided into two kingdoms, plant and animal. These are divisions made mainly for convenience since no sharp line of demarcation may be drawn between these groups. One may ask, "What is an animal or plant?" Such terms are used without much thought by both the layman and some scientists. Perhaps they are terms that cannot be defined but rather must be described at some length. Definition should be attempted with some knowledge of their origin. In early times when man applied the term plant to a group of living things and the word animal to another, there was little difficulty in using the terms. As knowledge accumulated, however, it soon became evident that there were intermediate forms which could be classed in neither group if all of their characteristics were considered. When the terms plant and animal were introduced it was an easy matter to distinguish between a tree or plant and an animal. The discovery of bacteria and other microorganisms, however, caused difficulty. In order to prevent a chaotic condition scientists usually attempt to systematize and group their data and information. Our forefathers in microbiology asked where bacteria would be placed in the plant or animal kingdom. The question is not an especially important one with which to spend a great amount of space but some profit will result if the student gives it a little thought.

Haeckel's Proposal.—Haeckel, a German biologist, who proposed the creation of a new kingdom to be called *Protista*, suggested placing in this kingdom those microscopic forms and lower forms of life which are grouped with difficulty in the two older kingdoms. This proposal involved the bacteria, yeasts, molds, algae, protozoa, and similar microorganisms. These organisms are said to be intermediate between the animal and plant kingdoms or to possess characteristics and properties which would seem to place them with either the plants or animals. Haeckel's proposal did not enjoy wide acceptance and Haeckel himself changed the limits of the group in some of his later writings and finally gave it up. As far as helping to settle the arguments about the location

of bacteria, etc., it would be of little value. It would probably increase the confusion since scientists would then have to decide with regard to three kingdoms instead of two.

Characteristics of Plants and Animals.—As stated above, the arrangement of living beings into groups called kingdoms is mainly for convenience and is, perhaps, the first step in their classification. The characteristics for separating plants from animals are probably adequate for the more highly developed forms but quite inadequate for the lower forms where there is overlapping in structure and function. The older zoologists recognized the existence of forms of life intermediate between plants and animals. These were called *zoophytes* or plant-animals. They are now placed with the animals, even though they possess some plant characteristics. In any attempt to classify living organisms, intermediate forms cause confusion. They are forms possessing characteristics of the larger groups which prevent placing them satisfactorily. Interesting discussions of this subject will be found in books on general biology.¹

DISTINGUISHING CHARACTERISTICS OF THE TWO KINGDOMS

Plants	Animals
1. Storers of energy	1. Liberators of energy
2. Plant cells have marked cell walls of cellulose	2. Animal cells may be devoid of cell walls, or have walls slightly developed
3. Root hairs and stomata absorb H ₂ O and gases; no digestion	3. Have an alimentary canal in which digestion takes place
4. Motion of liquid in wall ducts; Sugars descend in the walls of the inner bark	4. Circulatory system of heart, blood and blood vessels
5. Plants use CO ₂ and H ₂ O and nitrates. Liberate O ₂ . Organic matter built up. Chemosynthetic.	5. Metabolism: Animals use organic compounds, liberate CO ₂ and H ₂ O and urea. Organic matter destroyed. Chemoanalytic.
6. Well-developed vacuoles.	6. Vacuoles absent or slightly developed
7. Nucleoproteins containing a pentose	7. Nucleoproteins containing hexose.

General Method of Arranging Living Organisms.—The science of classification of living beings, whether plant or animal, is known as taxonomy. Only the general phases of the question will be considered here. This is necessary in order to give a proper setting for our discussion of

¹Hertwig, R. A Manual of Zoology (translated by J. S. Kingsley), Henry Holt & Co., New York.

microorganisms. It is necessary to have classifications and systematic arrangements of facts and data. Without such attempts it would be difficult for students to grasp the subject or to know when new facts were reported. The early philosophers recognized this need. Durant tells us that Aristotle believed every good definition to have two parts; one assigned an object to a class or group whose general characteristics were also its own; the second indicated wherein the object differed from other objects in its class. The various terms used in classification, classes, orders, families, etc., are really definitions.

Kingdoms.—This is the first separation made among living organisms. All forms of life belong either to the animal kingdom or the plant kingdom. As stated above, a proposal to form a third kingdom was not received with favor.

Phyla.—The next division of living organisms is into phyla (Sing. = phylum). Phyla, therefore are in a way sub-kingdoms. They include organisms which are alike in some major characteristic but differ in characteristics which may be used for further subdivision.

Classes.—The phyla are divided into classes. Living organisms having some salient characteristic in common are put into the same class.

Orders.—An order is a group of families. Names of orders end in *ales*.

Families.—A family is a group of tribes. Names of families end in *aceae* and sub-families in *oidea*.

Tribes.—A tribe is a group of genera. Names of tribes end in *eae* and of sub-tribes in *ineae*.

Genus (*plural Genera*).—The genus or generic name is comparable to our surname. It is the name which connects the species with the larger group. While practice differs, the name of the genus is usually italicized when printed and is written with a capital letter. The characteristics of the genus are more restricted than those of the family, tribe, etc. The members of a genus have many properties in common but differ in others on which separation is made between the species.

Species.—This term is used by all biologists in a general way but difficulties arise when they are called on to define it. Forms of life which resemble one another so closely that differentiation is difficult constitute a species. We may illustrate the point by taking some of the higher forms of life as examples since they are better known than the bacteria at this stage in our study of bacteriology. The *horse* may be regarded as belonging to a species, the *dog* to another, etc.; so also the *maple tree* belongs to a botanical species, the *rose* to another. Since the term species is so difficult to define, it is not surprising that confusion results when attempts are made to classify certain forms of life. One may ask the question, how much alike must two organisms be to belong to the same species or how unlike must they be not to belong to the same species? In order to obviate some of the difficulties inherent in the term species, some investigators (Darwin) use the word *varieties* for "sub-species" or groups within a species which seem to have certain salient characteristics in common.

Are Bacteria Plants or Animals?—This is a question which has caused some discussion and thought. Only in this way is it of any significance. It does not make much difference whether they are in the animal or plant kingdom so long as their fundamental properties are understood. Some bacteriologists class them with the animals, others with the plants. Could we arrive at an algebraic sum of their characteristics, they would probably fall into the plant kingdom. The tendency to class the bacteria with the plants is based on their ability to form spores and their chemosynthetic power. Bacteria are like some of the algae in several characteristics. Long, who gave this subject attention in his work with *Mycobacterium tuberculosis*, stated that a very useful purpose is served by the effort to fit all living beings into one or the other class so far as the evolutionary point of view is maintained; attention is thus kept focused on the root of all biological problems. Long could not find satisfactory data among either the morphological or physiological characteristics; consequently he sought chemical data. He did his work with *Mycobacterium tuberculosis* and, therefore, his conclusions may not be applicable to all bacteria. They are, however, of interest. Long found that the tubercle bacillus contained a nucleic acid of the animal type and no nucleic acid of the plant type.

Fungi.—The lower *Thallophyta* which produce a thread-like vegetation, called a mycelium, are called *fungi*. The group is an indefinite one including many different types of lower plants. They are especially characterized by the absence of chlorophyll, which fact predicts some of the more important characteristics. The lack of chlorophyll prevents fungi from using the energy in sunlight. They must resort to chemical energy, or that produced by chemical decompositions; thus, in order to secure the energy which nature has stored up in such large molecules as sucrose (cane sugar), dextrose (grape sugar), the protein molecule, the fat molecule, etc., fungi have become known as analytic microorganisms in that they decompose great amounts of organic matter.² Fungi are both parasitic and saprophytic. As stated above, many of the fungi are thread forms, although this is by no means a constant characteristic. Each thread in the mycelium is called a hypha (*plu.* = hyphae.) These hyphae may be segmented or septate or non-septate. Reproduction takes place by means of spores.

Arrangement of Contiguous Plant Forms.—Conn (1912) gave the following outline of the plant kingdom:

² See Lotka, A. J., Elements of Physical Biology. Williams & Wilkins Co., Baltimore, Md., Chapter 24, The Energy Transformations of Nature, for an interesting and pertinent, but somewhat mathematical, discussion of the energy transformations of living organisms.

Phylum I. THALLOPHYTA: plants without distinction of root, stem, or branch.

Sub-phylum 1. ALGAE: thallophytes possessing chlorophyll: including unicellular forms, pondweeds, seaweeds, etc.

Class I. *Diatomaceae*: the diatoms.

Class II. *Cyanophyceae*: the blue-green algae.

Class III. *Chlorophyceae*: the green algae.

Class IV. *Phaeophyceae*: the brown algae.

Class V. *Rhodophyceae*: the red algae.

Sub-phylum 2. FUNGI: thallophytes without chlorophyll: bacteria, yeasts, molds, etc.

Class I. *Schizomycetes*: the bacteria.

Class II. *Saccharomycetes*: the yeasts.

Class III. *Phycmycetes*: the alga-like fungi.

Class IV. *Ascomycetes*: the sac-fungi.

Class V. *Basidiomycetes*: the basidio-fungi.

Phylum II. BRYOPHYTA: the moss-like plants.

Class I. *Hepaticae*: the liverworts.

Class II. *Muscineae*: the mosses.

Phylum III. PTERIDOPHYTA: the ferns and their allies.

Class I. *Filicales*: the true ferns.

Class II. *Equisetales*: the horse-tails.

Class III. *Lycopodiales*: the club mosses.

Phylum IV. SPERMATOPHYTA: the seed-bearing plants.

Sub-phylum 1. GYMNOSPERMAE: the cone-bearing plants, pines, hemlocks, etc.

Sub-phylum 2. ANGIOSPERMAE: flowering plants.

Class I. *Monocotyledons*: endogenous plants.

Class II. *Dicotyledons*: exogenous plants.

Arrangement of Animal Forms: Conn arranged the animal kingdom as follows:

Division I. PROTOZOA: unicellular animals.

Class I. *Rhizopoda*: animals with naked protoplasts and pseudopodia.

Class II. *Infusoria*: animals with cilia, flagella, or tentacles, and usually a mouth.

Class III. *Sporozoa*: parasitic animals, producing spores and having a metamorphosis.

Division II. METAZOA: many-celled animals with a differentiation of cells.

Phylum I. PORIFERA: animals with no distinct mouth, but many incurrent openings: the sponges.

Class I. *Calcarea*: with a skeleton of calcareous spicules.

Class II. *Non-calcarea*: with a skeleton of silicious or horny spicules, or none.

Phylum II. COELENTERATA: animals with a mouth, but without an anus and with no body cavity.

Class I. *Hydrozoa*.

Class II. *Syphozoa*: sea-nettles.

Class III. *Actinozoa*: corals, anemones.

Class IV. *Ctenophora*.

Phylum III. ECHINODERMATA: radiate animals with complete alimentary canal and a body cavity.

- Class I. *Asterioidea*: starfishes.
- Class II. *Ophiuroidea*: brittle stars.
- Class III. *Echinoidea*: sea-urchins.
- Class IV. *Crinoidea*: sea-lilies.
- Class V. *Holothuroidea*: sea-cucumbers.

Phylum IV. PLATYHELMINTHES: flat, unsegmented worms.

- Class I. *Cestoda*: the tapeworms.
- Class II. *Trematoda*: the flukes.
- Class III. *Turbellaria*: the planarians.

Phylum V. NEMATHELMINTHES: round, unsegmented worms: round worms, threadworms.

Phylum VI. MOLLUSCOIDEA.

- Class I. *Polyzoa*: sea-mats, corallines.
- Class II. *Brachiopoda*: lamp-shells.

Phylum VII. ANNULATA: the segmented worms.

- Class I. *Chaetopoda*: bristle-footed worms.
 - Sub-class A. *Polychaeta*: with many bristles.
 - Sub-class B. *Oligochaeta*: with few bristles.
- Class II. *Hirudinea*: leeches.
- Class III. *Archannelida*.
- Class IV. *Gephyrea*.
- Class V. *Chaetognatha*.

Phylum VIII. MOLLUSCA.

- Class I. *Pelecypoda* or *Lamellibranchia*: bivalves, clams, oysters, mussels.
- Class II. *Gastropoda*: univalves, snails.
- Class III. *Amphineura*: many-valved: chiton.
- Class IV. *Cephalopoda*: with long arms: squids, cuttle-fishes.

Phylum IX. ARTHROPODA: with jointed feet.

- Class I. *Crustacea*: crabs, lobsters, barnacles.
- Class II. *Onychophora*: centipedes.
- Class III. *Myriopoda*: millipedes.
- Class IV. *Hexapoda*: insects.
- Class V. *Arachnida*: spiders, scorpions, etc.

Phylum X. CHORDATA: animals with a notochord.

Sub-phylum, ATRIOZOA: body cavity opening to the exterior.

- Class I. *Urochorda*: tunicates or sea-squirts.
- Class II. *Cephalochorda*: amphioxus.

Sub-phylum, VERTEBRATA: animals with a vertebral column.

- Class I. *Pisces*: fishes.
- Class II. *Amphibia*: frogs, toads, salamanders.
- Class III. *Reptilia*: lizards, snakes, turtles, alligators.
- Class IV. *Aves*: birds.
- Class V. *Mammalia*: mammals.

The Classification of the Fungi.—A brief outline of the botanical kingdom has been presented in an earlier paragraph. The second sub-phylum was divided into five classes of which *Phycomycetes*, *Basidiomycetes*, and *Ascomycetes* are of interest in connection with the molds.

Phycomycetes.—Phycomycetes possess the general characteristics of fungi, which have been discussed above. They are thread forms generally without cross walls (non-septate), and are consequently frequently called algal fungi. Sexual phenomena are exhibited in this group in the formation of zygospores.

Ascomycetes.—Ascomycetes include fungi the reproductive units or spores of which are formed within a membrane called as ascus. Different numbers of spores are formed. The true yeasts, *Aspergilli*, etc., are *Ascomycetes*.

Basidiomycetes.—Fungi called *Basidiomycetes* reproduce by the formation of basidia. Asexual spores are usually formed, although chlamydo-spores have been observed. The mycelium is septate.

Fungi Imperfecti.—The imperfect fungi or “fungi imperfecti” are separated from other fungi by the lack of well-defined fruiting bodies. Fungi which cannot be grouped with *Phycomycetes* or *Ascomycetes* are classed with Fungi imperfecti. Some imperfect fungi are *Oidium*, *Monilia*, *Endomyces*, *Torula*, *Mycoderma*, etc. It will be seen later that these organisms differ in characteristics. Some of them are discussed at different places later on in this book.

Distribution of the Bacteria.—Bacteria are widely distributed over the surface of the earth. There are, however, a very few places where they are not found. On account of this it is not unreasonable to attribute a very important rôle to them. They are probably no more important, though, than other forms of life since all living beings seem to have a part in nature's plan.

Normal Habitat.—Some bacteria are more commonly found in certain places than others. Such materials as milk and water are said to possess a “normal flora” because certain species are more liable to be found in them. The lactic acid bacteria, for instance, are commonly connected with milk and dairy products. Such groups of bacteria are often spoken of as the milk flora, water flora, soil flora, etc. It is quite a difficult matter to distinguish between the normal habitat and the temporary habitat for most of the common bacteria. However, with some of the uncommon species such as *Gonococcus*, or *Dialister pneumosintes*, a respiratory pathogen, the normal habitat is quite well known.

Temporary Habitat.—In contrast with those species which are especially adapted to a certain environment and which are usually expected when this is examined bacteriologically, there are those forms which are chance forms or temporary inhabitants. Bacteria are widely distrib-

uted and are present in or on most materials. It is not often easy to separate the temporary forms from the normal or regular forms.

Where not Found.—Even though we have stated that bacteria are very widely distributed, yet there are some places where they are not usually present as in the deep layers of the soil or in arid soils. This may be explained in different ways. It has often been stated that the body fluids and tissue of healthy animals are sterile. Recent data, however, seem to indicate that bacteria may, at times, be found in tissues of animals which seem to be in good health.

Rôle of Bacteria in Nature's Plan.—A little thought will soon convince a student that nature is a very delicately equilibrated system. Each group of organisms seems to have a definite function and may be necessary for the existence or growth of other groups. The bacteria are very active and a discussion of their metabolism will show that they form large amounts of metabolic products in proportion to body weight. The following processes may be mentioned as examples of the useful rôles of bacteria in nature.

Putrefaction.—Many complex substances such as proteins become useless and would be in the way were it not for the bacteria which split them into simpler substances. The carcasses of dead animals and plant structures, for instance, are attacked by bacteria and soon reduced to simple compounds. The chemical changes brought about by bacteria in putrefaction will be discussed later on in this book under the nitrogen cycle.

Fermentation.—This phenomenon, discussed also in other places in this book, is best known in connection with the preparation of fermented beverages. It has, however, a broader meaning since it may be involved in many important changes in nature. Much of the plant débris is fermented to soluble compounds which disappear and thus do not accumulate. An unhappy condition would result on the face of the earth if this were not the case. The fermentation changes are usually a part of the carbon cycle since the compounds fermented are mainly carbon compounds.

Under these two headings may probably be mentioned most of the desirable chemical reactions which form the bases for the great micro-biological industries. In many of these, man has not introduced a new reaction but has merely given certain bacteria an opportunity for doing in a much larger way what they do all the time in a small way.

Bacteria may play a rôle in furthering the normal development of animals and plants. A great number of experiments have been carried out to determine whether plant and animal life is possible without bacteria. This question will be discussed in some detail later on. It is

mentioned here as a part of the idea that bacteria have an important rôle in the general scheme in nature.

Scope of Microbiology.—The term *microbiology* in the broad sense includes the study of all microorganisms such as bacteria, yeasts, molds, algae, etc. A microbiologist, then, is an individual who studies such microorganisms. *Bacteriology* is the study of bacteria, and a bacteriologist, therefore, would be one who studies bacteria. Giltner has recently proposed the term “microbiology” as a term “relating to the biology of the small forms of life.” This proposal has considerable merit. The term *microbe* in the French means microorganism and probably has somewhat the same meaning in English. *Germ* is a term probably synonymous with the term *bacterium*. All of these terms are used by writers for designating various microorganisms. The field of microbiology is then a broad one and the term microbiologist is more fitting for the person who works with bacteria and related microorganisms than is the term bacteriologist.

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CHAPTER III

THE CELL

Many of the important functions of both macro- and microscopie organisms may be interpreted best by considering them as single cells. For instance, the function of metabolism and the enzymes connected therewith become more easily understood when studied in this manner. Since the single-celled microorganisms are mainly those with which the microbiologist has to deal, it is fitting that we consider the single cell before taking up the bacteria, yeasts and molds. Many of the facts of metabolism become more easily understood when they are interpreted on this basis. One is able to study the functions of single cells more easily than those of multicellular organisms. The cell, then, may be looked upon as the basic unit of life.

History of Cell Theory.—We need not use much space with this topic for it is discussed fully in several texts on cytology. While some early workers, when reporting their observations with the microscope, presented drawings containing structures which might be regarded as cells, Hooke, in 1665, was the first to report intelligently the “little boxes or cells distinguished from one another” in cork. The final argument on the existence of cells centers around the studies of two men, Schleiden and Schwann. They are generally regarded as the real founders of the cell theory although, according to Locy, the studies of Schwann were much more comprehensive. Locy believed that Schwann should have the credit for the cell theory and that Schleiden merely assisted him in his work. Schleiden and Schwann placed great importance on the cell wall, and believed it to be a necessary part of the cell. This idea, however, had to be given up when cells without walls were discovered. Schwann and his colleague also explained the formation of new cells by a crystallization process and not by division. Hugo von Mohl and Naegeli showed that a new cell was formed from an old one and that cells originated only in this manner and not by crystallization.

Plant and Animal Cells.—Distinctions between plant and animal cells, though often made on the basis of structure, are probably not definite. As has been stated elsewhere the early biologists recognized intermediate forms between plants and animals to which they gave the name zoophytes. This might indicate that the character-

istics of plant and animal cells might also intergrade. However, some differences may be mentioned. Wilson stated that animal cells are generally characterized by slight development of the membrane; plant cells, on the other hand, show well-developed cell walls. Wilson did not consider this difference to be of great significance. Vacuoles are also much more prominent in the cells of higher plants. In animal cells they are absent or obscure. Plastids are found in plant cells and centrosomes are absent.

PROTOPLASM

This term was coined by Hugo von Mohl in 1846 for the semi-fluid contents of plant cells. It is derived from the Greek *Protos* = first, and *plasma* = formed substance. Before this, Dujardin, a French biologist, in 1838 had used the name "sarcode" for the material in living organisms. Not much was discovered by these investigators with regard to the real character of this substance nor the similarity between animal and vegetable protoplasm. However, in 1861, Max Schultze proclaimed the similarity of protoplasm from different cells and from this date more rapid progress was made in its study. To-day we know that all protoplasm, regardless of the cells from which it comes, is quite alike. It has the same appearance and to a great extent the same properties.

The term protoplasm was sufficiently definite for the days when it was first introduced. However, as knowledge grew and more facts were discovered about the structure of the cell, the term lost its significance and others were introduced. The term *karyoplasm*, for instance, was introduced for the material within the nucleus and *cytoplasm* for the material outside of the nucleus. *Nucleoplasm* is also used for the substance in the nucleus.

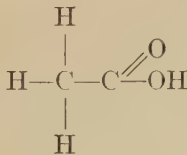
Chemistry of Protoplasm.—Protoplasm is not a chemical compound in itself but a mixture of compounds. The type and concentration of these compounds consequently varies. Besides the chemical compounds which may be regarded as normal there may be transitory compounds such as food material and the various products of its metabolism in the cell. There may also be certain chemical compounds in the cell laid down as reserve products such as those of sulfur and iron, fat, etc. The amount of these compounds is determined by the conditions under which the cell is propagated. Under conditions where there is plenty of food we might expect the maximum concentration of various constituents, while under conditions of starvation, they might be exhausted. A dozen elements may usually be found while a few others are present at times. We might also point out that chemical examinations on living matter are difficult to interpret.

One of the outstanding characters of protoplasm is its water content. While the water content of different types of protoplasm may vary the limits of variation are not wide. It plays an important rôle in maintaining the turgidity of the cell, carrying in the food and removing the waste products.

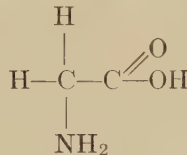
Salts are also present in protoplasm. Larson, who worked with bacteria, reported two kinds of salts—fixed salts and free salts. The former were bound up with the cell tissue while the latter could be removed by water. The salts along with the water probably maintain the proper osmotic pressure.

Protoplasm is also said to be in the colloidal state or to contain substances in this state. Findlay stated that 90 per cent of the organic matter of cells and tissues of the human body is made up of colloids. The term colloid was introduced by an English physicist, Graham, and applied to substances which would not diffuse through a parchment. Substances which would diffuse through such mediums were called crystalloids. Thus Graham recognized two states of matter rather sharply separated. To-day the line of demarcation has become less distinct and the terms denote only different states of matter.

Structure of Proteins.—Much of the material in protoplasm is protein in nature. Proteins are very complex chemical compounds built up from certain other chemical compounds called amino acids. These amino acids as the name indicates are organic acids with an —NH_2 (amino) group. We may take acetic acid as an example of a simple organic acid. One of the hydrogen atoms may be replaced by

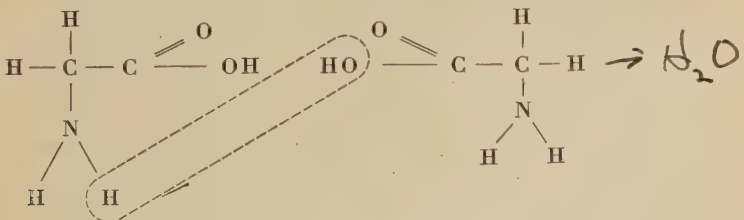


Acetic acid

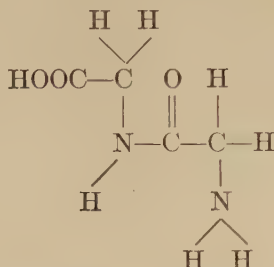


Amino-acetic acid or glycine

an —NH_2 group giving amino-acetic acid or glycine, the simplest amino acid. There are about twenty amino acids, all of which may be combined to form the proteins. The type of union may be shown by letting two molecules of amino-acetic acid react and become united as they would if we were starting to make a protein. The hydroxyl (—OH)



group of one molecule reacts with a hydrogen (H) of the amino group of the other molecule to form water (H_2O). This then unites these two amino acids as follows:



This gives what is known as a dipeptide. When several amino acids are thus linked, they form compounds known as polypeptides. Proteins are very complex polypeptides. The possibilities of complexity may be realized from the fact that the twenty or so amino acids may be linked in an almost infinite number of ways.

General Properties of Protoplasm.—*Movement*: Two kinds of movement are recognized in protoplasm. One is a real translocation of the cell and the other is known as a streaming of the granules in the protoplasm. These granules may be seen to move about the cell.

Irritability.—This characteristic is well shown by the fact that many agents are known to affect protoplasm. Heat, cold, chemicals, etc., all have decided influence. Heat is more harmful than cold. The characteristic of irritability forms the basis for many methods for controlling microorganisms.

Reproduction.—The ability of protoplasm to reproduce itself and grow is its outstanding property. Chemists are able to go far towards synthesizing compounds which are found in proteins and may sometime be able to synthesize a typical protein. However, they have not been able to endow any of these complex compounds with *life*.

Metabolism.—This is a necessary characteristic, for protoplasm must be generated and kept in repair. It includes practically all of the changes which are concerned with the use of food by the organism.

Death.—This is indeed the final property of protoplasm. The term death is an unsatisfactory one to define. One dictionary defines it as "cessation of life." Such a definition is not very satisfactory since definition of the term life is just as elusive. Some biologists state that protoplasm never dies. They claim that since reproduction involves the division of cells, life is continuous. It is difficult to discuss and define death since it is equally difficult to define life. Lepeschkin has stated that life represents a sum of physiological phenomena which may be observed not only with the whole organism, but with each cell that

composes it. Death of multicellular organisms must proceed by degrees because not all cells would die at the same moment. Even with single cells, death may be a somewhat progressive phenomenon. Death is known to be concerned with alterations in the chemical and physical structure of living matter. The relatively recent developments in the field of colloids give an opportunity for partial explanation, perhaps, for the causes of death. The colloid structure of living matter is changed by death.

MORPHOLOGY OF CELLS

Each cell may contain a number of specific parts called organs. Some of these will be described briefly. Cells differ in size and shape. Some bacterial cells are known which are only about 0.2 micron in diameter, while other bacterial cells and some yeast cells are many times larger. Size is indeed a variable characteristic.

The Cell Wall.—The cell wall is not a necessary structure since some cells are known to be devoid of walls. The wall is probably a protective organ and it may undoubtedly influence many of the activities of the cell. Its chemical composition varies greatly. There are probably two layers on most cells. The outer one may assume a thicker, sticky consistency to form what is known as the capsule. Some cells are enclosed in a sheath.

Cytoplasm.—What we have said about protoplasm may also be applied to cytoplasm. The constitution of the cytoplasm has received much study. Several theories obtain for explaining its structure.

1. *The filar theory.*—According to this theory the cytoplasm is full of threads embedded in a ground substance.

2. *The reticular theory.*—This theory is that the threads in the cytoplasm, indicated by the filar theory, are connected end to end.

3. *The alveolar theory.*—This theory holds that the ground substance of the cell is filled with droplets giving the appearance of an emulsion.

4. *The granular theory.*—According to this theory the ground substance is filled with granules.

Wilson, probably the greatest authority on cell structure, holds that the cell substance is homogeneous in its first state. Later filar, globular and granular structures may appear.

The Cell Nucleus.—This is a very important organ in the cell. The substance in the nucleus, *karyoplasm*, is distinguishable from the cytoplasm by its greater coagulability in some of the organic acids, acetic and chromic. Consequently these acids are used for the demonstration of the nucleus.

The nucleus contains a liquid in which are the other parts. These are *chromatin* and *achromatin*. About the nucleus is a membrane. The chromatin is one of the most important parts of the cell. The *nucleolus* is also contained in the nucleus.

Shape of the Nucleus.—The nucleus exists in different shapes in different cells. In certain cells the entire cell contents seems to be made up of nucleoplasm. In other cells the nucleus is diffused throughout the cell. The cells of higher animals have more complex nuclei.

Rôle of the Nucleus.—One of the important rôles of the nucleus is in cell division. It probably carries the hereditary factors of the cell to its progeny. The nucleus also plays a rôle in the regeneration of tissue. When certain protozoan cells are divided into two parts, only that part which contains the nucleus can regenerate itself.

This suggests the question whether an enucleate cell can exist. After knowing the importance of the nucleus in cell activities, one is almost compelled to answer the question negatively. This question is discussed again under cytology of the bacterial cell.

Centrosome.—This is a very small body in the protoplasm. For some time, on account of its size, it was overlooked. As will be briefly discussed later in this chapter, the centrosome, also, plays an important rôle in cell multiplication.

Vacuoles.—These are pockets or vesicles in the cytoplasm filled with liquids. They seem to function in regulating the water content of the cell.

Organs of Locomotion.—Many cells of unicellular organisms possess organs of locomotion with which the cell is moved from place to place. Some cells, however, are able to move without them since they resort to a corkscrew-like motion or a snake motion. Other cells are able to produce an oscillatory motion. Then, in the protozoa there are the pseudopodia for motion. The most characteristic organ for locomotion however, is the *flagellum* or the *cilium*. Flagella are whip-like appendages on the cell which are lashed back and forth in the medium. Cilia have a similar structure and function but are possessed in greater numbers covering most of the cell.

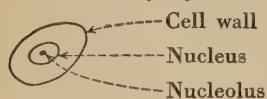
Other Organs in the Cell.—Besides the organs which have just been mentioned there are others which are of interest to students of cytology. Plastids are found especially in plant cells. They vary in shape, size and number and are believed to be synthetic infunction. Wilson stated that they were areas of specific chemical transformation producing characteristic products such as starch, fat, chlorophyll, etc. The different plastids have been named from the products which they are supposed to make. Amyloplastids make starch, chloroplastids make chlorophyll, etc. Granules of different kinds are also found in cells. Metachromatic

granules are found; their purpose has been the subject of much discussion.

MULTIPLICATION OF CELLS

All cells reproduce or multiply by division. There is no other method.

Direct Division.—This is also referred to as *amitosis*. It is simpler than mitosis since only one cell is involved—the one which is about to divide. The division of the cell is preceded by a division of the nucleus. The nucleus changes shape—usually elongates; a constriction appears which finally results in two nuclei being formed. These separate and a constriction appears in the cell. This progresses until the cell divides into two cells each of which has a nucleus. The process may be shown schematically by the following figures.



1. Cell in resting stage.



2. The nucleolus divides into two parts.

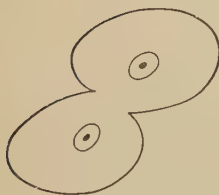
This phenomenon has not been observed in all cells.



3. Constrictions develop on the nucleus.



4. The nucleus divides leaving two nucleoli.



5. Constrictions begin to form in the cell wall.



6. The constriction becomes deeper and deeper until finally the cell divides into two cells.



Indirect Division.—This is also called *karyokinesis* or *mitosis*. It is more complicated than direct division which has just been discussed. Metcalf devised a very useful chart showing the several stages in mitosis. This is reproduced in Fig. 11. Metcalf discussed the chart as follows:

The process of cell division is represented as a cycle. The cycle is represented by five phases; each phase, excepting the prophase, is represented by stages which are intended to represent an early stage and a late stage in each phase.

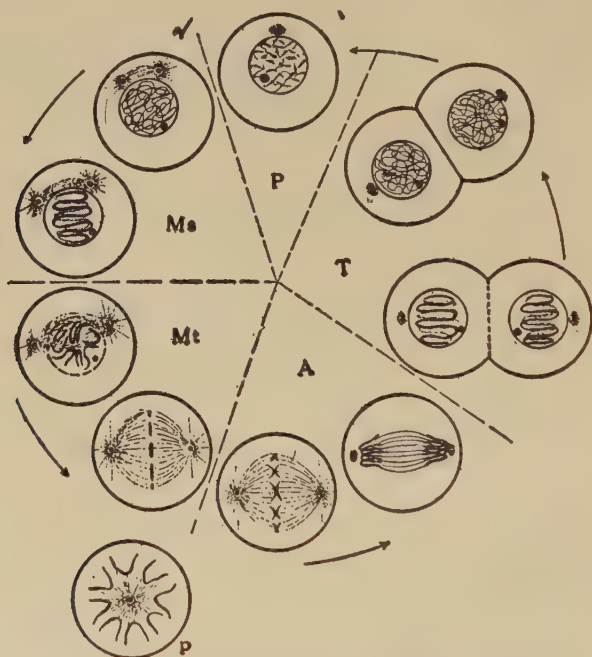


FIG. 11.—Showing the Various Steps in Mitosis. (After Metcalf, 1924.)

See text for discussion.

The prophase, *P*, is limited to the resting (mitotically speaking) nucleus; enclosed in a nuclear membrane with its chromatin material in the form of granules as usually described; with a large nucleolus; and a centrosphere containing two centrosomes. Emphasis is always placed upon the facts that while we speak of this as a resting nucleus we are speaking mitotically and that metabolically this is the active phase of the cell and that as far as the life of the cell is concerned it is a very long period and further that it is really the end of the cell's existence as a cell and not its beginning.

The term mesophase, *Ms*, is introduced to designate that phase of the process of mitosis described as spireme thread formation. The other char-

acteristic thing in this phase is that the centrosomes have commenced to separate. The mesophase is divided into two stages. In the first stage the spireme thread is long, slender and much coiled. In the second stage the thread has shortened and thickened and has been thrown into a definite number of loops, each loop corresponding to a future chromosome.

The metaphase, *Mt*, is characterized by the chromosomes. The chromosomes are represented as the broken loops of the short-looped spireme thread. Another character is the disappearance of the nuclear membrane. In the first stage the chromosomes are represented as being scattered in the cell, while the centrosomes are about 90° from each other. In the second stage the centrosomes have reached their polar positions, 180° from each other, and the chromosomes have arranged themselves at the equator. Both equatorial and polar views, *p*, of this stage are shown for the sake of clearness. Attention is usually called to the disappearance of the nucleolus during this stage. Mitosis may be said to have reached its climax with this stage and all the rest of the process may be described as a process of reconstruction of the chromatin material.

The anaphase, *A*, means that time during mitosis when there are daughter chromosomes in the cell. It starts with the first indications of the splitting of the chromosomes and ends with them arranged at the poles. The first stage shows the beginning of the splitting and the second stage shows the chromosomes pulled to the poles and the division of the cytoplasm started. The centrosomes have usually divided by this time in preparation for the next mitosis, and the nucleolus has usually reappeared.

The telophase, *T*, may be described as the daughter spireme phase. In the first stage we have short thick-looped daughter spireme threads formed apparently by the growing together of the ends of the looped chromosomes. This stage is exactly comparable with the second stage of the mesophase save that we are now "back-tracking" and have daughter spireme threads instead of a single thread. The nuclear membrane has reformed and the cytoplasmic division is usually complete. In the second stage we have long daughter spireme threads which are comparable to the first stage of the mesophase and the interesting process of mitosis is all but finished, for there remains but the single step, the transformation of the thread into scattered granules, and we are back with daughter prophase stages ready to start the whole process over again. And while in the minds of the older students who have learned the old nomenclature we may have appeared to add to the confusion by introducing new terms and by limiting old terms in a new way, and to have simply run round in a circle and arrived nowhere, yet my experience with beginning students leads me to believe that there is some little merit in it.

Sexual Phenomena.—These occur in many cells although there are some which are said to be devoid of them. They have been recently reemphasized for the bacteria. In those microorganisms such as the yeasts among which they have been especially studied, cell fusion results in a revivification of the cell as shown by more rapid reproduc-

tion or spore formation. Guilliermond has done much to elucidate sexual phenomena among the yeasts. The two units which are involved are spoken of as *gametes*; a copulatory canal is formed between the two gametes through which the contents of one cell is poured into the other. The male gamete is smaller usually than the female gamete and pours its contents into the female gamete. When the gametes are equal in size the process is spoken of as *isogamy*; if unequal *heterogamy*. Involved in these sexual unions are the usual nuclear changes so well known for the larger single-celled organisms such as the protozoa.

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CHAPTER IV

MORPHOLOGY OF BACTERIA

A number of difficulties arise in the study of the morphology of bacteria. While some of them greatly complicate our attempts and have caused serious differences of opinion, much has been discovered about these small microscopic organisms. The first difficulty which presents itself is the small size of the cell. Many of the facts regarding morphology and physiology of bacteria, and the related microorganisms, have come from observations on a large number of bacteria. This method of studying living organisms has its disadvantages. These become more obvious when we think of attempting to determine the detailed characteristics of a cow by observations upon a group of one or two million cows, even though they be of the same breed and species. Much safer information is possible in the latter case by studying one or a few separate individuals. Such a situation would be the ideal one for the bacteriologist but it is almost impossible for him to make such observations on a large scale. He must be content, under ordinary circumstances, with observations on a great many cells at the same time. Another disadvantage is the fact that these small single-celled microorganisms are quite liable to change in characteristics when grown in the laboratory away from their natural habitat. New generations follow one another with great rapidity and if new characteristics are assumed they will be picked up quickly.

SHAPE AND SIZE OF BACTERIAL CELLS

Shape.—Bacteria differ markedly in shape. This characteristic was one of the first to be used for classification. In general there are three shapes commonly recognized by bacteriologists:

Coccaceae: Round or spherical cells.

Bacteriaceae: Rod or cylindrical-shaped cells.

Spirillaceae: Spiral-shaped cells.

Since these names indicate the shape of the organisms, we will meet them again under the discussion of Classification.

Having established these large groups of bacteria on the basis of their general shape, let us see how and on what basis these groups are subdivided. We may first give attention to the *Coccaceae*. These are round cells and are subdivided into groups on the basis of the space relationship of the cells to one another. The names to indicate this are:¹

- Micrococcus*: Single, isolated round cells.
Streptococcus: Chains of round cells, some very long, some short; like a string of pearl beads.



FIG. 12.—Showing Chains of *Streptococcus lactis*. India Ink Preparation. (After Orla-Jensen.)

A. Cells from a broth culture, three days at 10° C. B. Cells from a broth culture, 10 days at 10° C.

- ✓ *Diplococcus*: Two round cells situated close together with the adjacent surfaces slightly flattened. The two cells look something like two coffee beans placed together.

- ✓ *Staphylococcus*: Irregular groups of round cells—bunch of grapes appearance.

- ✓ *Sarcina*: Round cells dividing in three directions of space generally giving cubical packets.

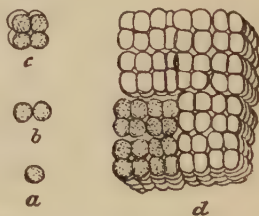


FIG. 13.—*Sarcina ventriculi* Goodsir. From the contents of a diseased stomach. (From Schmidt and Weiss after Zopf.)
a, single cell; b, the same after division in one direction; c, the same after division in two directions; d, packet of cells.

¹ The following terms for certain round cells are taken from the newer classifications. They have not, however, been universally accepted. Other names will be found in the chapter on Classification.

Leuconostoc: Round cells which adhere one to another.

Neisseria: Round cells existing in pairs; frequently pathogenic.

Gaffkya: Round cells existing as tetrads.

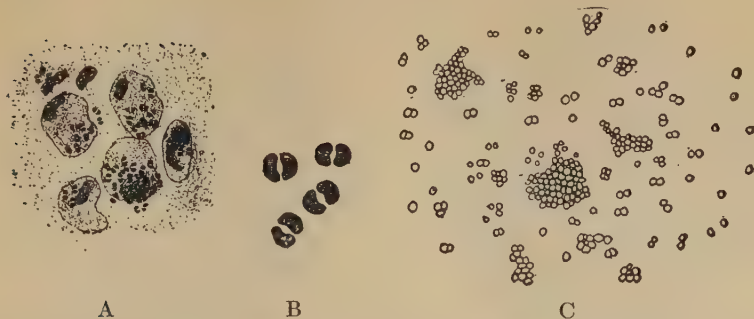


FIG. 14.—*Neisseria gonorrhoeae* (*Diplococcus gonorrhoeae*). A. Showing the Organism inside of White Blood Corpuscles. (From Schmidt and Weiss.)

B. The Isolated Cells. C. *Staphylococcus aureus*. Isolated and Groups of Cells.

The group of cylindrical cells is not subdivided in the same manner as the round cells. We cannot use the location of the cells in relation to one another as a basis of subdivision. Some systematists have separated the group on the basis of the presence or absence on the cells of organs of locomotion. Others—and this is the method we may tentatively accept—subdivide the group on the presence or absence of endospores.²

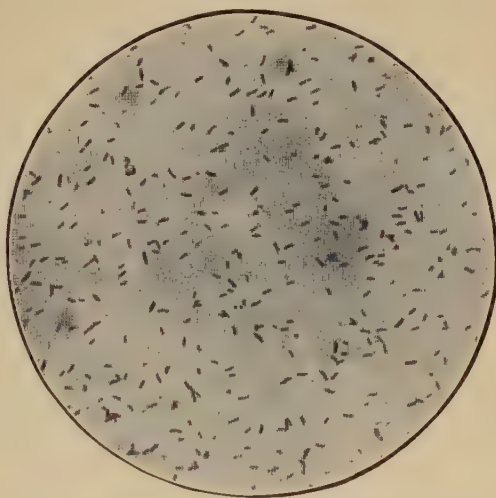


FIG. 15.—Photomicrograph showing Rod-shaped Bacteria. *Denitrobacterium thermophilum*. (After Ambrosz.)

Bacterium: Rod or cylindrical cells without endospores.

Bacillus: Rod or cylindrical cells forming endospores.

Pseudomonas: Rod cells with polar flagella.

² The following are some of the names from the new Classification which are given to some of the rod forms. Other names will be found in the chapter on Classification.

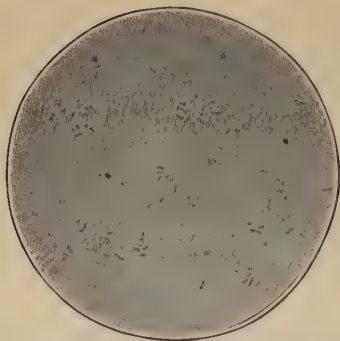
Chromobacterium: Rod-shaped organisms producing pigments.

Flavobacterium: Rod-shaped bacteria forming yellow to orange pigment.

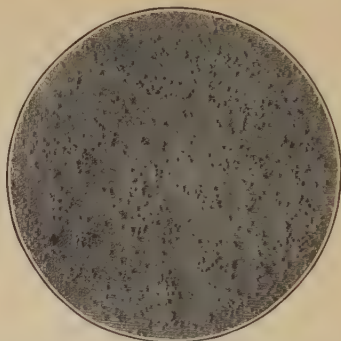
Lactobacillus: Slender rods producing lactic acid.

Erwinia: Motile rods, frequently pathogenic for plants.

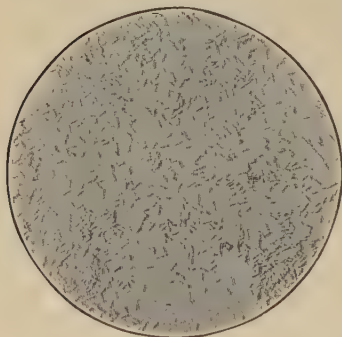
PHOTOMICROGRAPHS OF BACTERIA (Different species.)



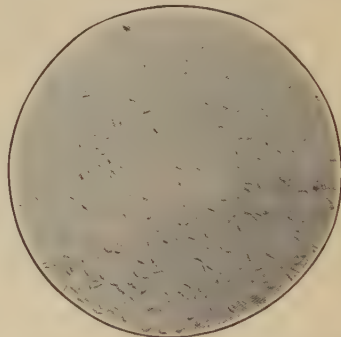
1.—*Aerobacter cloacae* (*Bacillus cloacae*). Cells from Glucose Broth after Twenty-four Hours at 30° C.



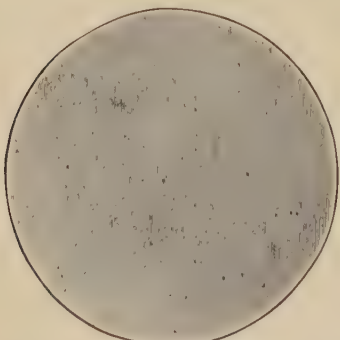
2.—*Escherichia communior* (*Bacillus coli communior*). Cells from Glucose Broth after Twenty-four Hours at Room Temperature.



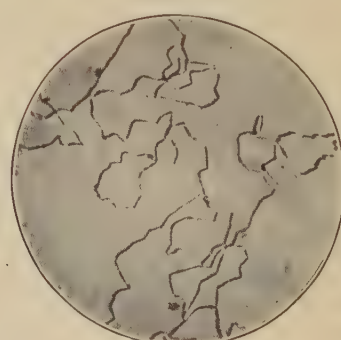
3.—*Achromobacter viscosum* (*Bacillus lactis viscosus*). Cells from Glucose Broth after Twenty-four Hours at Room Temperature.



4.—*Bacillus niger* (*Bacillus lactis niger*). Cells from Plain Agar after Two Days at 30° C.



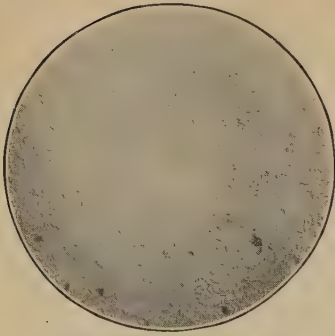
5.—*Lactobacillus acidophilus* (*Bacillus acidophilus*). Cells from Milk after Twenty-four Hours at 37° C.



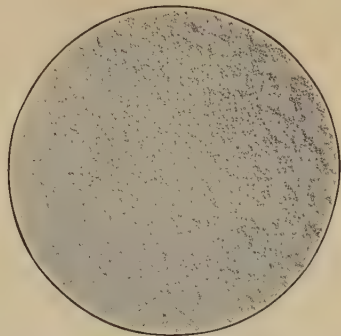
6.—*Bacillus subtilis*. Adhesion Culture after Twenty-four Hours at 30° C.

PLATE II

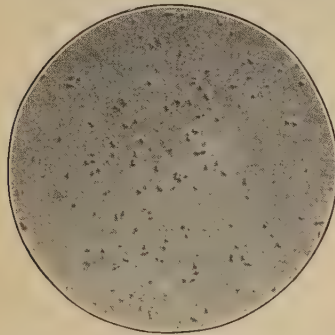
PHOTOMICROGRAPHS OF BACTERIA AND OTHER FUNGI



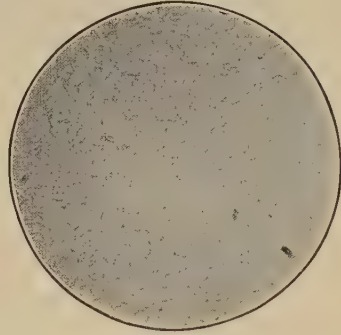
7.—*Pseudomonas fluorescens* (*Bacillus fluorescens*). Cells from Plain Broth after Three Days at Room Temperature.



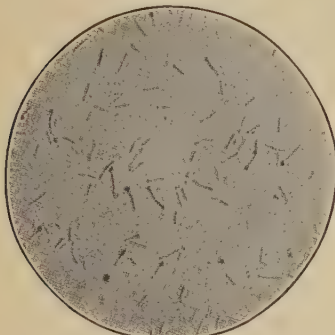
8.—*Staphylococcus aureus*. Cells from Plain Agar after Three Days at Room Temperature.



9.—*Erwinia amylovora* (*Bacillus amylovorus*). Cells from Glucose Broth after Twenty-four Hours at Room Temperature.



10.—*Spirillum rubrum*. Cells from an Agar Slant.



11.—*Clostridium tetani* (*Bacillus tetani*). Cells from Glucose Broth after Three Days at 37° C. Note the spores in the ends of some of the rods.



12.—*Oidium lactis*. Cells from Lactose Broth. After Three Days at Room Temperature.

The third large group which we mentioned, the *Spirillaceae*, are subdivided according to differences in the degree of the bend or curve in the cell. The following names are used to indicate these sub-groups:

Microspira (*Vibrio*): Short rigid rods slightly bent.

Spirillum: Long rigid cells.

Spirochaete: Long flexible cells.

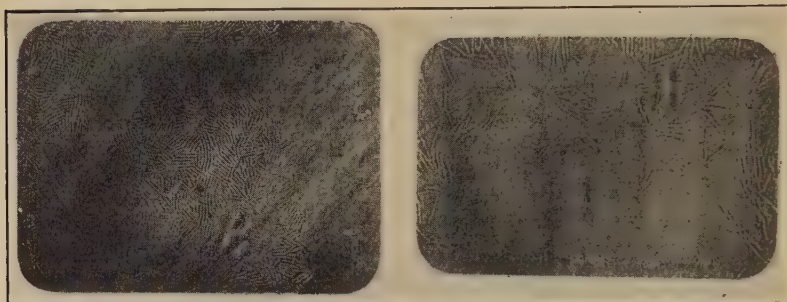


FIG. 16.—Showing Rod-shaped Cells in the Margin of an Anthrax-like Colony.
(After Hagan.)



FIG. 17.—(After Migula).

A. *Spirochaete plicatilis*; B. *Spirochaete Obermeieri*. A schematic illustration showing the shape of the cells.

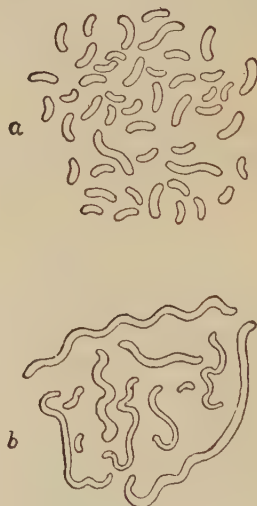


FIG. 18.—*Vibrio comma* (*Microspira cholerae*). (After Migula.)

a. Single cells; b. Long spiral-shaped cells with a few so-called involution forms.

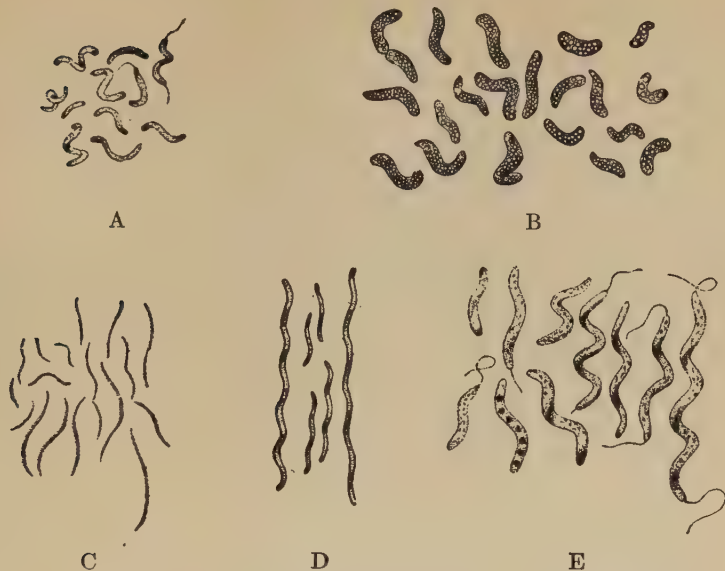


FIG. 19.—Showing Various Spiral-shaped Bacteria. (After Warming.)

A. *Spirillum undula*; B. *Spirillum Rosenbergii*; C. *Spirillum serpens*; D. *Spirillum tenue*;
E. *Spirillum volutans*.

Size.—Bacteria are known as very small organisms—so small that they are invisible with the naked eye. This fact delayed their discovery until after the time when special instruments were devised. The microscope opened up this world of microorganisms for study. Their discovery necessitated a new unit of measurement since to express their dimensions in the older units would cause a great waste of time and greater possibilities of error. Consequently microscopists introduced a new unit, the micron, which was defined as 1/1000 of a millimeter or about 1/25,000th of an inch (1/25,400th to be exact). The Greek lower case letter *mu* (μ) is taken as the symbol.

An average rod-shaped bacterium might be $0.5\mu \times 3-4\mu$, although the exact size depends on many factors such as the species, conditions of nutrition, age, etc. As the name indicates, *Clostridium giganteum* is an unusually large cell the diameter often being 2μ and the length $8-10\mu$.

Clostridium butyricum is also an exceptionally large organism often



FIG. 20.—*Thiospirillum rosenbergii*. (*Spirillum Rosenbergii*).
(After Warming.)

Showing cells with sulfur granules.

reaching 10 microns in length by 1 micron in diameter. *Bacillus megatherium* is another very large organism. Probably the smallest bacteria known are the coccus-forms or the coccoid organisms.

There are also other ways of expressing the small size of bacteria.³ Conn in his "Practical Dairy Bacteriology" states that a drop of milk may contain as many as 100,000,000 and a particle the size of a pin-head 8,000,000.

Size and Weight of Bacteria.—While bacteria vary widely in size and weight many of them are within rather narrow limits. The average bacterial cell is, perhaps, about 0.5 micron in diameter and from 1 to 1.3 microns in length. On both sides of this average are microorganisms which are distinctive for either their small or large size. *Dialister pneumosintes* (*Bacterium pneumosintes*), a microorganism isolated from certain respiratory infections, is one of the smallest organisms that has been described. Its size is given as 0.15 micron in diameter and 0.3 micron in length. *Spirillum colossum* has cells which were reported as 3.5 microns long. *Clostridium giganteum*, as the name indicates, is a very large microorganism which may vary from 0.75 to 2.0 micron in diameter and 8 to 10 microns in length. The size of the cells varies with a number of factors. For instance, it is known that cells grow after division so that the age is an important factor. The state of nutrition, as would be expected, also has some influence.

MacNeal, Latzer and Kerr determined the number of cells of bacteria required to yield a gram of dry bacterial substance. Using *Escherichia coli* (*Bacterium coli*) in one experiment, 55×10^{11} cells (5,500,-

³ Microscopists and physicists who work with very small beings and particles have had to use new units of measurement. It might be useful or interesting, at least, to define several such units.

Micron = μ = 1/1000 of a millimeter, or 1/25,400 of an inch.

Millimicron = $\mu\mu$ = 1/1,000,000 of a millimeter.

Ångström unit = 1/10,000,000 of a millimeter.

Objects measured in microns are visible with a microscope. An ordinary microscope cannot distinguish particles of less than 0.13 micron in diameter. Very fine dust particles have a size of about 2 microns. Particles less than 4 microns in diameter show the phenomenon of Brownian movement. Having defined the units of measurement commonly used it might be interesting to see the sizes of different cells and particles.

Hydrogen molecule.....	0.067 $\mu\mu$
Water molecule.....	0.113 $\mu\mu$
Chloroform molecule.....	0.800 $\mu\mu$
Bacteriophage.....	20 to 40 $\mu\mu$
Starch grains.....	5 to 7 μ
Red blood corpuscles.....	7.5 μ

000,000,000) were required to yield 1 gram of bacterial substance on the dry basis. Other experiments yielded data which were not widely different. Jordan in his contribution to that interesting book, "The Nature of the World and of Man," stated that it would require 12,000 cells of *Eberthella typhi* (*Bacterium typhosum*) to reach an inch and that a cubic inch would hold about 9,000,000,000,000 such cells.

Rubner reported the specific gravity of bacteria to be between 1.038 and 1.065. Another investigator placed the limits at 1.120 and 1.35. Kendall has stated that 1,600,000,000 cells of *Escherichia coli* (*Bacterium coli*) would weigh a gram. Winslow stated that

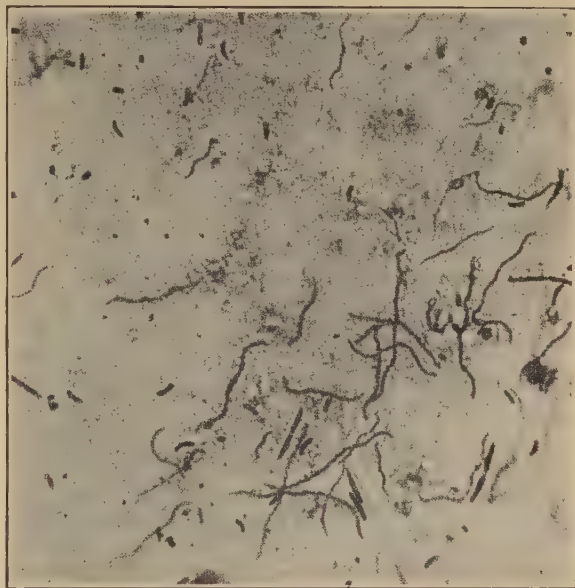


FIG. 21.—Typical *Spirillum* Organisms of Trench Mouth; Oil-immersion Photomicrograph of a Cover Slip taken from a Patch on the Tongue. (After Bloodgood, 1927.)

400,000,000 cells could be packed into a space occupied by a grain of sugar. Such statements help one to visualize their small size.

Higher Bacteria.—Many forms of life intergrade into one another. We have learned that there is no sharp line of demarcation between the plants and animals and that once having selected definitions for the term plant and animal, many intergrading forms are encountered which have characteristics of both of these groups. So, also, we find difficulty in establishing satisfactory limits for the group of organisms known as bacteria. On both sides of this group, we find contiguous forms which have characteristics like some of those of the true bacteria. On what may be called the upper or higher side of the true bacteria, we have the *higher bacteria*. These are forms of life midway between the bacteria and higher forms of life such as algae, protozoa, etc. Some of the *Spirochaetes* are classed as higher bacteria. It might be profitable to discuss briefly the structure and functions of some of the higher bacteria

to determine how they are connected with the true bacteria. In some cases there is a union of cells to give a more complex structure. These forms are often called Trichobacteria or *Chlamydobacteriaceae*.

Shape.—The higher bacteria may exist as single cells or in masses. They are usually composed of straight threads; branching is uncommon.

Reproduction.—The species is reproduced either by spores from various cells in the thread or by special units formed by the cells at the tip of the thread. The latter are like conidia.

Sheath.—Frequently the cells composing the thread are surrounded by a structure called a sheath. The cells, then, exist in a sort of tube. Frequently some special compound is formed and deposited in the sheath as a reserve substance. This is the case with iron in *Crenothrix*. With the sulfur bacteria it is sulfur.

We may mention some of these forms which have special significance.

Streptothrix.—These organisms have characteristics of both bacteria and molds. They reproduce

by spore formation and have branching filaments. *Nocardia* is another name for this group.

Crenothrix.—This fungus has branched filaments with no sulfur granules.

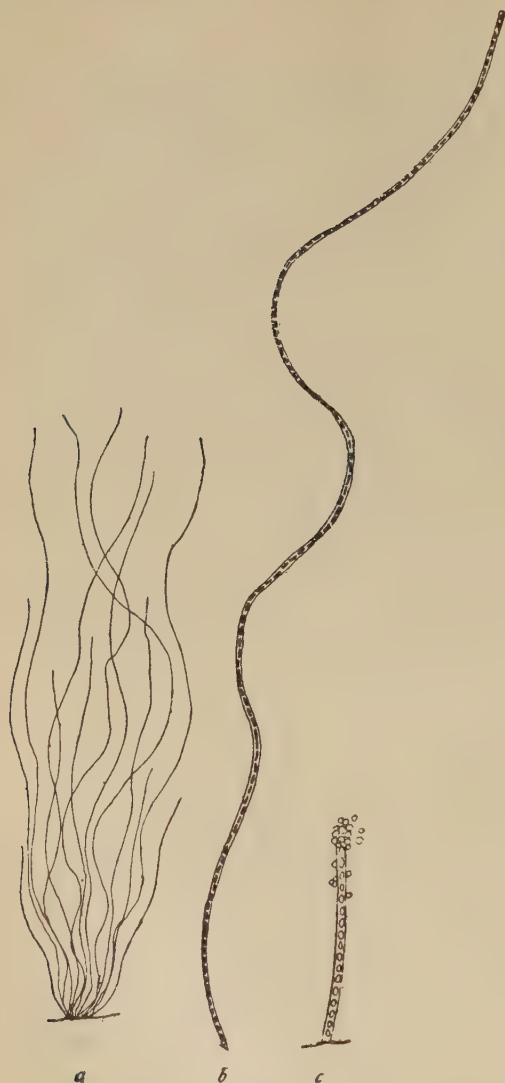


FIG. 22.—*Leptothrix hyalina* (*Streptothrix hyalina*.)
(After Migula.)

It possesses a sheath in which cells may be seen to move about. This sheath is usually colored brown by the deposition of iron compounds. *Crenothrix* is usually found in iron-containing waters, and may cause trouble in clogging water mains or imparting an undesirable appearance to drinking water.

Cladothrix.—The filaments are branched and may, also, contain iron deposits. The best-known species is, perhaps, *Cladothrix dichotoma*. So-called swarm spores are formed by the opening of the sheath.

Lower Bacteria.—On the other side of the true bacteria, there may exist living organisms which are much smaller—probably too small to be seen by ordinary microscopes. They are called lower bacteria. Such a state of affairs is to be expected. However, we do not need to rely entirely on conjecture. Much evidence has been accumulated to support the existence of lower bacteria.

Filterable Viruses.—One group of organisms falling under the general heading, Lower Bacteria, are known as filterable viruses. Since lower bacteria are too small to be seen with the ordinary microscope, one may ask, then, how they are known to exist. Their existence is proven by a different technic. Physicists are able to prepare very fine filters from diatomaceous earth, stone, etc., and to measure the size of the pores or openings. They are able to prepare these filters with such fine pores that the true bacteria cannot pass through but remain on

the filter. Then, if by means of certain special tests, the filtrate be shown to contain an agent which will bring about some change, we have proven that it came through the filter, and knowing the size of its pores we know that the organism must be smaller.

In order to make this clearer, let us take a specific example. A number of years ago, Beijerinck found that the mosaic disease of the tobacco leaf was due to an agent which could not be seen and which would pass through very fine filters. When this filtrate from an emulsion of diseased leaves was put on healthy tobacco leaves, these leaves sickened and showed all the symptoms of the disease. What steps did Beijerinck have to carry out in order to rule out the possibility of error in his conclusions? We may tabulate them as follows:

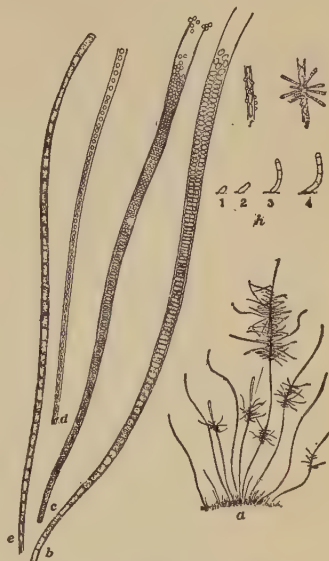


FIG. 23.—*Crenothrix polyspora*.
(After Migula.)

1. Secure some diseased tobacco leaves and emulsify them in some fluid.
2. Filter this emulsion through a sterile stone filter.
3. Examine the filtrate culturally and microscopically in order to prove the absence of true bacteria or other forms of life visible with ordinary microscopes.
4. Having proved the absence of such forms of life inoculate healthy tobacco leaves to reproduce the disease.

The separation of viruses into groups on the basis of filterability is not entirely satisfactory. It has been shown that other factors besides size of the particle may influence filterability. The dye Victoria blue will not pass through a Berkefeld filter while Congo red, as acid dye, will. The type of filter whether it is made of diatomaceous earth, plaster of Paris, etc., seems to be important. Consequently filterability may not be satisfactorily demonstrated at times. Experiments of this nature are also complicated by the fact that the filters may vary and, in some cases, allow the passage of ordinary bacterial cells. The appearance of growth might erroneously cause one to conclude that a filterable virus or stage in the cycle of the microorganism had been demonstrated.

Many diseases in which the causal microorganism has not been demonstrated have been attributed to filterable viruses. It is interesting to note, however, that as information is amplified some of these diseases are regularly taken from the group said to be caused by filterable viruses and placed in the group caused by organisms with a definite shape which can be seen with the microscope.

Bacteriophage.—An agent which is able to cause disintegration of bacteria cells in broth or on agar was reported by d'Herelle about 1917. Other investigators at previous times had noticed phenomena which may have been due to bacteriophage. They did not, however, study the cause of these phenomena. Action of bacteriophage is evidenced by the clearing of turbid suspensions of bacterial cells or by the appearance of clear areas on agar plates heavily seeded with bacteria. Investigators are agreed that the action of this agent may be reproduced almost at will and that the agent may be isolated from various substances. However, there is great disagreement over the nature of the agent. D'Herelle believes that it is a living filterable organism; he called it *Bacteriophagum intestinale*. Another investigator claims that the bacteriophage is a stage in the life cycle of another organism. Others have classed the agent as an enzyme. D'Herelle, who has done much work with the agent, believes that it is a living agent the cells of which are too small to be visible by modern microscopes. He believes that the bacteriophage preys on the bacteria causing them to be de-

stroyed. The bacteria may be infected by these invisible parasites in the same manner that bacteria infect larger organisms. A great number ⁴ of papers as well as a number of books have been written on this subject. The most tenable theory at present is that bacteriophage is not a living agent.

Variability in Shape and Size.—This is a topic over which there is a great difference of opinion. As a standard for comparison one must accept some form of the microorganism as the normal form and explain deviations from this according to any of the several possibilities. Bacteria seem to be very active forms of life. They reproduce very rapidly, decompose a large amount of food for their body weight and size and consume or transfer a large amount of energy. One need not be surprised that they are somewhat labile in shape and function.

Mono- vs. Pleomorphism.—Many of the early bacteriologists were pleomorphists, believing that bacteria could change from round cells into rods, etc. Fischer expressed it in this manner: "The pleomorphists maintained that a coccus did not necessarily remain a coccus its life long, but it could, under certain conditions, stretch itself and assume the shape of a bacillus, that this again could become curved and change into a vibrio, to return again later on to the coccus form that it commenced with. Words like *Micrococcus*, *Bacillus*, *Vibrio*, *Spirillum* were in the eyes of the pleomorphists worthless designation of transient changes in shape." These early pleomorphists introduced confusion if not chaos into the subject of cell shape. After this the pendulum swung back to monomorphism where it remained for some time. Considerable evidence seems to be accumulating to give support to a new pleomorphism—that bacteria may exist in more than one shape. The modern conception, however, involves a more orderly bacterial world with one form following another in regular sequence in what have been called life cycles or life histories.

Mutation Forms.—The word "mutation" comes from the Latin verb *muto*, meaning "I change"; a mutation form, then, is a form which has changed from one which has been taken as the normal. It is not always easy to describe the normal form and consequently there is just as great difficulty in describing so-called mutation forms. Bacteria also differ greatly in their ability to mutate. This change in shape is probably due to some stimulus in the environment of the organism. Some of the stimuli of mutation have been studied, such as the influence of temperature and the composition of the medium. Hansen, in his

⁴ Marshall, M. S. The Bacteriophage. Amer. Jour. Pub. Health, 16 (1926), 180-182.

study of the acetic-acid bacteria, studied the influence of temperature on the appearance of mutation forms.

Hansen studied especially the effect of temperature on morphology. By transferring cells from a medium at 34° C. to one at 40°–40.5° C., a very apparent alteration in shape was noted even in a short time. The rods became longer and then grew into long threads.

These predominated. When these long threads were placed again at 34° C., they began to swell and in other parts to break up. This dismemberment continued until the long threads were gone.



FIG. 24.—*Acetobacter aceti* (*Bacterium aceti*). (After Hansen.) Showing Filamentous Cells with Unusual Shapes from Culture on Wort and Similar Media. (After Hansen.)

Involution Forms.—Naegeli used the term involution forms for so-called deformed, aberrant forms like those seen in other groups of living beings. The term involution has just the opposite meaning to evolution. The former indicates a regression in development or function while the latter means a progression. The term in the past has been used to cover a multitude of deviations in shape from a form considered to be the normal. Some of these deviations now have better explanations. The term has probably served as a convenient refuge for explanation of forms which differed from what was accepted as the normal. The early bacteriologists believed that involution forms were especially characteristic of old cultures.

The abnormally shaped cells which occur in fresh young cultures of certain bacteria are far more difficult to explain. Bacteriologists ordinarily assume that young cultures of bacteria held at the optimum temperature are most active and normal in every respect. However, with certain bacteria such as *Azotobacter*, it is sometimes almost impos-

ible to explain the appearance of these abnormal forms. The early bacteriologists believed that involution forms were especially characteristic of old cultures.

sible to secure cultures free from those abnormal, aberrant forms commonly referred to as involution forms. Under such optimum conditions, is it right to consider them as decrepit, diseased, or weakened forms? Such observations cause one to wonder just how useful the term involution form is, whether it should be retained with its old meaning or replaced by newer terms which more adequately fit the facts.

Life Cycles.—The fact that certain bacteria, even when cultivated under the most favorable conditions, showed the presence of abnormal forms, has caused several investigators to study them. One of the most

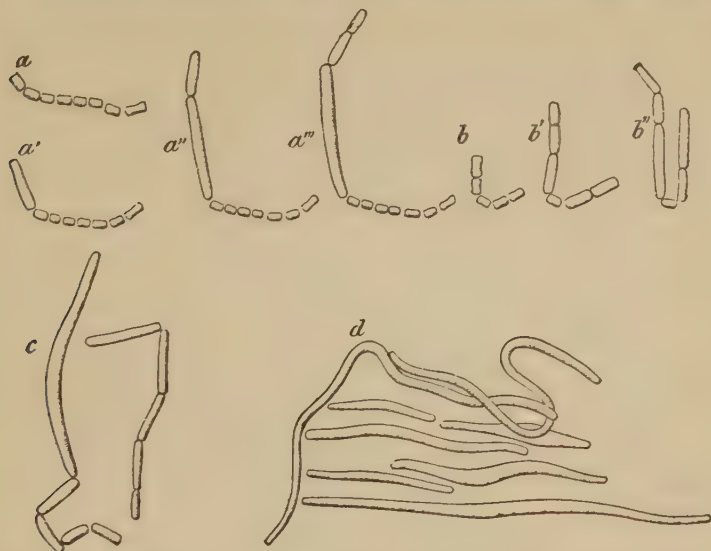


FIG. 25.—*Acetobacter pasteurianum*. (*Bacterium pasteurianum*.) Showing the Morphological Change from Short Rods to Long Threads. (After Hansen.)

a, A chain of eight short rods; a'–a'', the same after six, ten, and twenty hours; b, chain of five short rods; b'–b'', the same after five and nine hours; c and d, after ten and twenty-one hours.

interesting theories of modern bacteriology has resulted from these studies. Löhnis and Smith, after studying a number of common bacteria suggested that these forms passed through a number of different stages to make up a life cycle or series of stages in which one form may follow another in approximation to some definite order. Life cycles are known for higher organisms. The malarial parasite, for instance, has a complicated life cycle involving several hosts. The butterfly, also, has a life cycle which is perhaps more striking on account of the great variation in shape and habitat of its several stages.

Despite the fact that, as soon as we come up the evolutionary line of living beings to forms which are easily studied, even certain microscopic

forms, we find rather complicated life cycles, the theory of Löhnis and Smith has met with considerable opposition. Whether the statement of these authors is true or not, one readily recalls the opposition which many of the truths that are so firmly established to-day and generally accepted had to meet. It is not the purpose of this paragraph to present this theory as an established truth, although there are many data from the experiments of other authors which support it, nor to pass over



FIG. 26.—*Vibrio comma* (*Microspira cholerae*). Various Shapes of Cells.
(After Migula.)

I, Single Cells; II, Spirillum-shaped Cells; III, Various Shaped Cells; IV, So-called Involution Forms.

it as a fantastic idea, but to present it for thought. Löhnis and Smith summarized their first paper as follows:

A comparative study of 42 strains of bacteria has shown that the life cycles of these organisms are not less complicated than those of other micro-organisms. As representatives of practically all groups of bacteria have been tested and all, without exception, behaved essentially in the same manner, in all probability analogous results may be expected with all species of bacteria.

All bacteria studied live alternately in an organized and in an amorphous stage. The latter has been called the "sympylastic" stage, because at this time the living matter previously inclosed in the separate cells undergoes a thorough mixing either by a complete disintegration of cell wall, as well as



FIG. 27.—A. *Corynebacterium diphtheria*. So-called Involution Forms; B. *Mycobacterium tuberculosis*. Involution Forms. (After Schmidt and Weiss.)

cell content, or by a "melting" together of the content of many cells which leave their empty cell walls behind them. In the first case a readily stainable, in the latter case an unstainable "sympylasm" is produced.

According to the different formation and quality of the symplasm the development of new individual cells from this stage follows various lines. In all cases at first "regenerative units" become visible. These increase in size, turning into "regenerative bodies," which later, either by germinating or by stretching, become cells of normal shape. In some cases the regenerative bodies also return temporarily into the symplastic stage.

Besides the formation of the symplasm, another mode of interaction between the plasmatic substances in bacteria cells has been observed, consisting of the direct union of two or more individual cells. This "conjunction" seems to be of no less general occurrence than the process first mentioned. The physiological significance remains to be studied.



FIG. 28.—Showing the Development of Bacteria to Bacteroids. (After Beijerinck.)

All bacteria multiply not only by fission but also by the formation of "gonidia"; these usually become first regenerative bodies, or occasionally exospores. Sometimes the gonidia grow directly to full-sized cells. They,

too, can enter the symplastic stage. The gonidia are either liberated by partial or complete dissolution of the cell wall or they develop while still united with their mother cell. In the latter case the cell wall either remains intact or it is pierced by the growing gonidia, which by come either buds or branches.

Some of the gonidia are filterable. They also produce new bacteria either directly or after having entered the symplastic stage.

The life cycle of each species of bacteria studied is composed of several subcycles showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions. The transformation of spore-free into spore-forming bacteria seems to be dependent on the conditions acting upon the symplasm and regenerative bodies.

The discovery of the full life cycles of bacteria may be helpful in many directions. Systematic bacteriology now can be established on a firm experimental basis. Physiological studies will win considerably in conformity and accuracy when connected with morphological investigations along these new lines. Several problems in general biology are brought under more promising aspects. Agricultural bacteriology and medical also will derive much benefit.

A summary of the information on shape of bacteria indicates a more orderly condition of the field than existed in the early days of the science. Instead of a mobile field wherein the transformations were determined by chance, we are, perhaps, approaching a belief that these changes follow one another in an orderly manner. This may make it necessary to alter some of our theories and classifications. It is not at all improbable that some of the present-day species are merely different stages in the life cycle of a few microorganisms. This might account, in part, for the application of different names to the same microorganism. Such a necessity should not cause worry since science should be founded on truth. If it is necessary to alter some of the older information on account of new discoveries, bacteriologists should be willing to do it.

STRUCTURE OF BACTERIA

Bacteria are such small organisms that there is much to learn about the internal structure of their cells. However, great progress has been made toward the solution of what at first seemed an insurmountable problem. Less definite information is available for bacterial cells than for yeast and protozoan cells. In a former chapter were briefly discussed some of the organs of a typical plant or animal cell. Now the same thing may be done for the bacterial cell.

Cytoplasm.—Bacterial cytoplasm is probably little different from that of all cells. It consists of protoplasm having the general properties

of this substance. Within the cytoplasm are the other organs which will be discussed below. Most of the studies which have been made on this substance have involved the use of very large bacterial cells. In 1889 Bütschli studied the structure of *Chromatium okenii*, in which he distinguished a central body and parietal layer. The central body, by means of hematoxylin, was reported to possess a structure shown in Fig. 29. Granules, to which he gave the name "red grains," were also seen in it. This central body was thought to be the nucleus and the parietal layer the cytoplasm. Bütschli's statements have been the subject for considerable discussion.

Cell Wall.—The cell wall of bacteria is a protective covering secreted by the cytoplasm. As far as is known all bacterial cells possess cell walls which give to the cell definite form and shape. The cell wall should be looked upon as a semipermeable membrane through which food and waste products pass in the general life processes of the cell.

The structure of the cell wall on certain large forms has been studied by a number of bacteriologists. Such studies necessitate the use of special methods to make it visible. Fischer used plasmolysis to separate the cytoplasm from the cell wall. After the cytoplasm had been drawn away from it, the wall appeared as a delicate membrane still intact. By means of such technic, Fischer called attention to the fact that the cytoplasm in bacterial cells was not connected to the wall and that the osmotic pressure in bacterial cells was half that in cells of the higher plants, since the bacterial cells required solutions only one-half as strong for plasmolysis. Fischer was unable to distinguish a finer structure in the cell membrane.

Capsules.—Some bacteria possess a gelatinous coating about the cell. This is either an excretory product which happens to be of such consistency that it is held about the cell or it is an outer layer and a constituent part of the wall. This is called a capsule. Not all species of bacteria seem to be provided with well-defined capsules. The material composing the capsule is of a gelatinous, mucilaginous consistency causing the cells of a capsulated organism to adhere and stick together. This

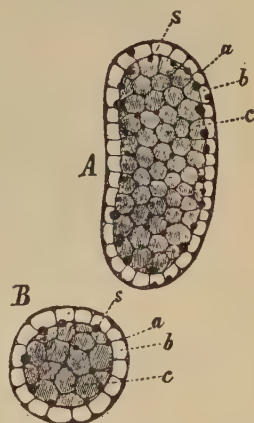


FIG. 29.—*Chromatium okenii*. (After Bütschli.)

A. Longitudinal Section; B. Cross Section. The cell was first killed and then freed from bacterio-purpurin and sulfur granules by solvents, and finally stained with hematoxylin. The reticulated structure of the central body (c), as also of the parietal layer (b) and the dark chromatium granules (s) Bütschli's red grains can then be detected.

gives what is spoken of as *zoogloea* formation, a phenomenon of considerable importance in the industries. Capsulated bacteria are responsible for outbreaks of ropy or slimy milk, ropy bread, etc. Capsules were first noticed by Friedlander on his *Pneumobacillus* in 1883.

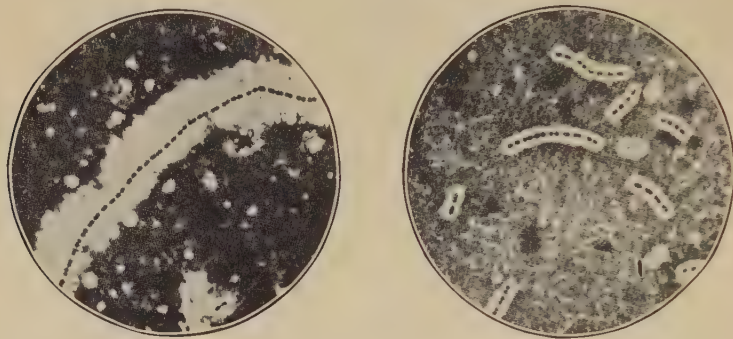


FIG. 30.—Showing Capsules on Cells of *Streptococcus cremoris*. (After Orla-Jensen.)

Several methods are available for determining the presence of capsules. The cultural method usually gives the first information that an organism possesses a capsule. When the organism is transferred from one medium to another, the growth often is viscid enough to pull out in short strings. Another method rests on the fact that the capsule resists

ordinary staining procedures, and consequently appears as an unstained halo about the cells. Chemical studies on the material in the capsule by the early bacteriologists indicated that it was identical with mucin.

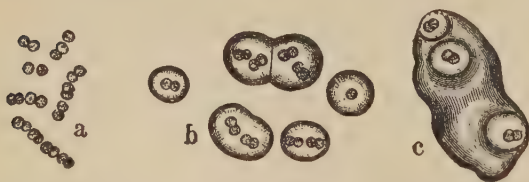


FIG. 31.—*Leuconostoc mesenteroides*. Showing the Zoogloal Masses and Capsules on the Cells. (After Schmidt and Weiss.)

Recently it has been reported to consist of gelactan. The contradictory data may, perhaps, be due to the fact that the chemical composition varies in capsules of different species of bacteria.

Organs of Locomotion on Bacterial Cells.—Some bacteria are provided with whiplash-like appendages with which they propel themselves through the medium. The names *cilia* or *flagella* (Plural) are given them: The latter term is most used for bacteria while the former is used for the protozoa. Since the number of flagella and their location on the cell are variable, bacteria may be differentiated on this basis. The adject-

tives used to denote the presence or absence of flagella, and their location on the cell when present, are:

Atrichous = absence of flagella.

Monotrichous = one flagellum on one end.

Lophotrichous = tuft of flagella on one end.

Amphitrichous = tufts of flagella on both ends.

Peritrichous = flagella all about the cell.



FIG. 32.—Flagella on Bacterial Cells. (After Migula from Schmidt and Weiss.)

A. *Spirillum rubrum* with lophotrichous flagella. B. *Pseudomonas syncyanea* with amphitrichous flagella.

It is quite probable that some bacteria are able to move about even though they do not possess definite organs of locomotion. There may be other means such as a contractile membrane by which motility is



FIG. 33.—Peritrichous Flagella. (From Migula after Schmidt and Weiss.)

A. *Eberthella typhi* (*Bacterium typhosum*); B. *Proteus vulgaris*.

accomplished. Motion may also be accomplished by a snake-like movement by which the cell propels itself through the medium. Other movements of protoplasm may also be responsible for motility.

In comparison with the higher animals bacteria move more rapidly.

Lehmann and Freid measured the distance traveled by several bacteria. It took *Eberthella typhi* (*Bacterium typhosum*) one second to travel 0.018 mm. The velocity of motion is dependent on many factors such as temperature, age, state of nutrition, etc., and specific data ought not to be applied to all species.

Brownian Movement.—A phenomenon which frequently complicates motility studies on bacteria was announced in 1827 by the botanist Brown. While he was examining fine materials such as pollen, under the microscope, he observed that they underwent a constant



FIG. 34.—Monotrichous Flagella. (After Migula from Schmidt and Weiss.)

A. *Pseudomonas javanensis*; B. *Microspira Cholerae*; C. *Pseudomonas pyocyanea*; D. *Pseudomonas macroselmis*; E. *Spirillum undulata*.

vibration, the degree of which increased as the particles became smaller. This phenomenon finally became known as Brownian movement, for which a number of explanations have, at times, been offered. It is now believed that this movement is due to the bombardment of the particle under observation by the molecules of the liquid in which it is suspended. This vibration is also apparent with bacterial cells and must be distinguished from real motility—a translocation from part of the microscopic field to another.

Nucleus.—The nucleus is an organ which has a very important rôle in cytology. It is probably the most important organ in the cell being concerned with reproduction and the carrying over to the progeny of the characteristics of the parent cell or cells. The question of the presence of a nucleus in bacterial cells is one over which there is much discussion. The situation may be analyzed by considering the several possibilities which have been suggested.

1. The bacterial cell is a very primitive one and is devoid of nucleus. By analogy, at least, there is little to support this view beyond the fact that a nucleus has not been as satisfactorily demonstrated in all bacterial cells as in the cells of higher microorganisms. Those who con-

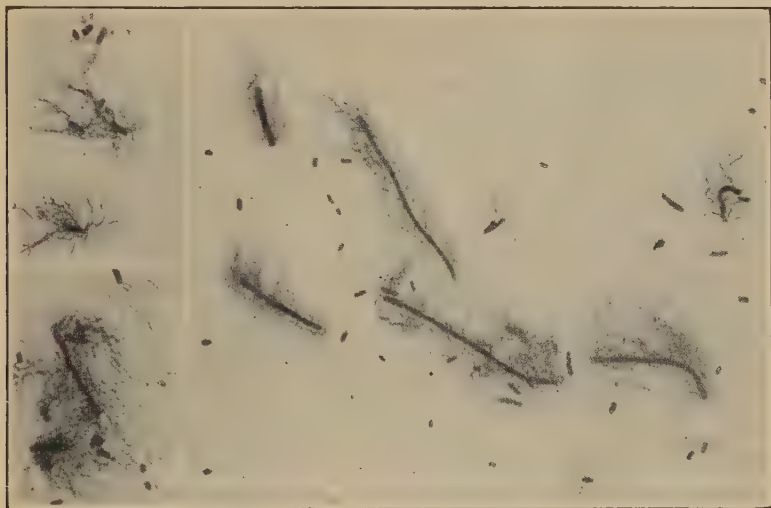


FIG. 35.—Photomicrographs Showing Flagella on Various Strains of *Proteus* Species.
(After Bucciante, 1926.)

tend that bacteria are enucleate explain the bodies reported as nuclei as resulting from the treatment given the cells by heat and stains. It is doubtful what the proponents of this view mean by the term primitive. Certainly a cell which can reproduce itself every 30 to 60 minutes, eat its own weight every hour or so, and bring about such a great amount of chemical change per unit of time, is not primitive.

In the study of biology we learn that the nucleus has great significance in the life processes of the cell and its existence is usually admitted. Is it reasonable, then, to cast doubt on its existence if it cannot be shown to be present in bacteria as convincingly as in yeasts, and protozoa, for instance?

2. A nucleus is present, but is diffused throughout the cell giving what we may speak of as a diffused nucleus. One of the most important supporters of this view was Bütschli.

3. A nucleus is present, and constitutes practically all of the cell interior.

4. A nucleus, like the nucleus in any typical cell, is present. This theory then implies that the bacterial cell is no different in constitution from other cells which are more easily studied. Let us consider the evidence from the laboratories of a number of different investigators.

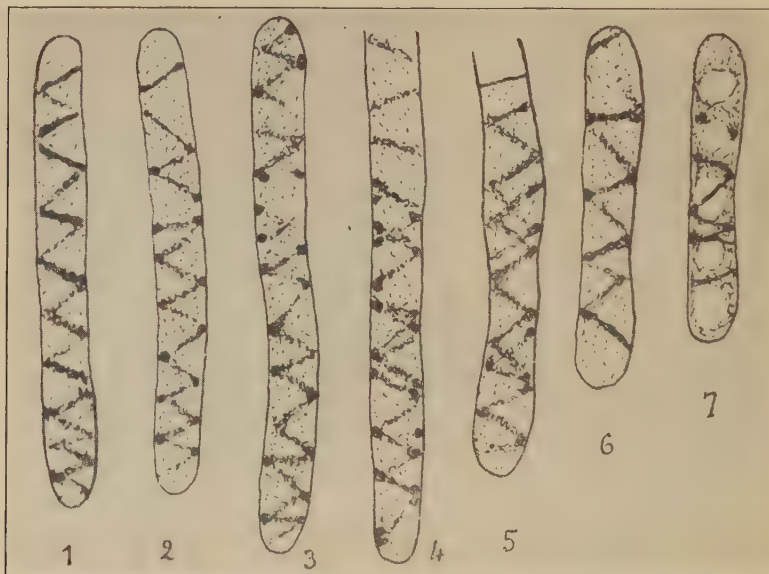


FIG. 36.—*Bacillus maximum bucchalis*. Showing the Development and Division (in 4 and 5) of the Chromidial System. (From Guilliermond after Swellengrabel.)

Bodies demonstrable by the ordinary nuclear stains have been reported by many. Dobell, one of the foremost students of microbial cytology, believes that bacteria are nucleate organisms.

Some chemical evidence may also be brought forward to support the presence of a nucleus. Mathews in his "Physiological Chemistry" states that the proteins of the cell nucleus are sharply differentiated from those in the cell cytoplasm. In the nucleus many of the proteins are nucleoproteins in which are nucleic acids. Mathews sums up the situation by saying that in the nucleus are found nucleoproteins whereas in the cytoplasm these are probably lacking. Accepting this statement as applicable to the bacteria, it may be stated that nucleic acids have been found in *Escherichia coli* (*Bacterium coli*) and *Mycobacterium tuberculosis*.

The author believes that the several lines of evidence strongly support the presence of a nucleus in bacterial cells. In coming up the evolutionary line of living organisms we find the presence of a nucleus admitted just as soon as we reach microorganisms that may be studied conveniently, protozoa and yeasts, for instance. The nucleus is satisfactorily demonstrated among these two groups. The cells of these microorganisms are considerably larger than bacteria and are more easily studied. With the bacteria, however, the cells are smaller and more difficultly studied. In this respect, it is quite interesting to note that even among the bacteria when the larger cells have been studied, the presence of a nucleus has been reported. When the smaller cells have been used, however, the evidence for the presence of a nucleus is not quite so satisfactory. Recalling the important rôle of the nucleus in cytology it is difficult to imagine a cell without this interesting organ. The summation of the several lines of evidence, chemical, staining, as well as circumstantial, justifies the conclusions that bacterial cells are nucleate. In some cases the nucleus may be diffused, in others, organized.

Sporulation.⁵—Various forms of life seem to enter stages where their life processes are markedly slowed up but probably not stopped. These are often referred to as resting stages. Certain higher animals hibernate in the winter. The encysted stage is also present among the protozoa; they remain in that condition until favorable conditions obtain. This makes it possible to distinguish two types of protoplasm, *vegetative* and *sporoplasm*. The vegetative protoplasm is the active growing protoplasm while the sporoplasm is that which is in the spore.

Formation of a Spore.—Those who have followed the formation of a spore in a bacterial cell have reported a concentration of protoplasm; this is eventually surrounded by a membrane which becomes the wall of the spore. This was well shown by Schaudinn with *Bacillus Bütschlii*. Nuclear changes have also been reported in the formation of the spore.

Spores may be located in the rod either in the end or center. In the former case they are spoken of as polar spores and in the latter as equatorial spores. Sometimes the spore is so large that it distends the cell

⁵ The term arthrospore was introduced by early bacteriologists for a sort of encysted state among the bacteria. This spore was not formed inside of the cell like the endospore. The entire bacterial cell was believed to assume the characteristics of a spore by forming a thick wall. The term has not had wide usage among bacteriologists but is frequently seen in some of the older texts. Löhnis has used the term for one of the stages in his life cycle of *Azotobacter*. In contrast with the term arthrospore as used by the older bacteriologists is the term endospore. The later spore was formed within the cell in the same manner as is known to-day. The separation of bacteria into arthrosporous and endosporous groups is not based upon good experimental evidence.

wall giving the cell a bulged appearance. Such a cell has been called a *Clostridium*. This cell shape has been characterized in different ways by the early bacteriologists. Some of these names are used in modern literature, such as drum-stick when the swelling is in one end, and spindle shaped when the swelling is in the middle.



FIG. 37.—Showing Cell Division and the Formation of Endospores in *Bacillus Bütchlii*. (After Schaudinn.)

Structure of the Spore.—The marked resistance of the spores to unfavorable agents would suggest that they might possess a cell structure quite different from that of vegetative cells. The cell wall is probably the first part which is of interest from this viewpoint. The spore wall consists of two layers. The cytoplasm of the spore probably differs from the



FIG. 38.—A. *Bacillus luxosus*, Burchard. (After Burchard.) B. *Bacillus simplex*, Gottheil (*Bacillus loxosporus*, Burchard).

cytoplasm of the vegetative cell in some way because it is often more refractive under the microscope. It is known to contain less water.

Number of Spores Produced.—Generally but one spore is produced by a cell. This is borne out by the fact that when an organism is found which forms more than one spore it is indicated in the name. For instance we have Kern's *Dispora caucasica* and *Bacillus inflatus*.

Properties of the Spore.—The spore is a very resistant unit in the life cycle of the cell. This marked resistance to a variety of unfavorable

agents may be explained in different ways, none of which may be the true explanation. It has been suggested that the cell wall is thicker and more dense, preventing the passage through it of the unfavorable agents. Another explanation has it that the spore is resistant on account of a lower water content. The death of bacteria, at least by heat, is probably due to protein coagulation for which water is very necessary. Consequently with less water in the sporoplasm, we would expect it to be more resistant.

On account of its significance in the food industries and sterilization, the resistance of spores to heat is, perhaps, their best-known characteristic. As would be expected, a marked difference exists in the heat resistance of vegetative protoplasm and sporoplasm. Brefeld reported that the spores of *Bacillus subtilis* required heating for 3 hours at 100°C. to destroy them, whereas vegetative protoplasm could be killed in 20 minutes. The spores of *Clostridium botulinum* are especially heat resistant. The spores of some strains will withstand between 4 and 5 hours boiling. Thermophilic bacteria also have heat-resistant spores. The high temperature optimum of thermophiles gives them a high thermal death point. This heat resistance causes these micro-organisms to be of marked significance in the canned-food industry. While we have singled out heat as the important agent to which spores are resistant, they are just as resistant to other agents. Some chemicals have no effect on spores and consequently cannot be used with safety for the destruction of spore-bearing bacteria.

Germination of the Spore.—Spores are supposed to remain dormant as long as they are under unfavourable conditions, to germinate when favorable conditions obtain. This process involves a renewed activity on the part of the protoplasm in the spore, an activity which results in the rupture of the spore wall. In a book of this nature we need not discuss the details of this process as they have been described for certain bacteria. There seems to be some uniformity as to the place where the spore wall is

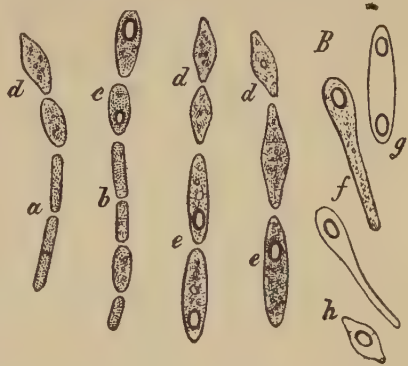


FIG. 39.—*Clostridium butyricum* (*Bacillus amylobacter*, van Tieghem). Showing Spore Formation. (After Prazmowski.)

a, b, Vegetative cells; d, spore beginning to develop; c-e, further development of spores; f-h, spores completely formed; a-f, granules stained blue with iodine; h, stained with the same reagent devoid of these granules; g, cell with two spores.

ruptured to permit the protrusion of the germ. In some organisms it is in the middle, giving what is called equatorial germination (Fig. 41).



FIG. 40.—*Bacillus parvus*, Neide (*Bacillus leptosporus*, Klein). (After Migula.)

Usually the spore envelope disappears but one or two species have been discovered on which the envelope remains. Another method of spore germination was described by Prazwoski with *Bacillus subtilis* (Fig. 42).



FIG. 41.—Equatorial Germination of Spores. *Bacillus subtilis*. (After Migula.)

Function of Spores in the Life History of the Organism.—This allows us to consider some of the reasons for spore formation. Several may be mentioned.

1. Spores are formed to carry the cell over an unfavorable period

caused by the lack of food, the accumulation of products of metabolism, etc. This, then, places on the spore the responsibility of perpetuating the species under certain conditions. Also, it means that only certain species are endowed with this ability, for many are known which do not form spores.

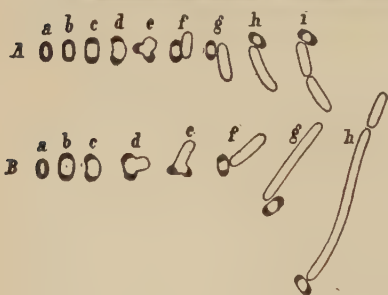


FIG. 42.—*Bacillus subtilis*. (After Prazmowski.)

a, Ripe spore; b, placed in a nutrient solution the refraction disappears; c, enlargement begins; d, the equatorial fissure is formed and the young germ begins to escape; e, in the upper row the central portion of the germ is just protruding, in the lower row one pole is already free; f, the young rod is free; g, it grows to its normal size; h, reproduces by division.

2. Spores are reproductive units.

There is little doubt about the spore having the ability to reproduce and perpetuate the species. After the spore has lain dormant for an indefinite period, favorable conditions will cause it to germinate, grow and reproduce a plant structure like that from which it originated.

While the spore, when placed under favorable conditions, will reproduce the species and thus function as a reproductive unit, the fact that not all bacteria form spores causes some confusion. It seems improper

to offer as a means of reproduction a process which is not enjoyed by all the members of a group.

Methods of Demonstrating Presence of Spores.—We will not attempt in this place to enumerate the details of bacteriologic technic but merely the principles on which some of the methods rest. Most of them are based on, or at least concerned with, the resistance of the spore to some agent.

Staining Methods: Spores are resistant to the dyes used for staining bacteria. Consequently, the sporoplasm remains almost colorless in contrast with the vegetative protoplasm which easily takes the color of the dye being used.

Heat Resistance: This method takes into account the great heat resistance of spores. A culture of an organism suspected of being a spore-former may be heated to such a temperature that the vegetative protoplasm would be destroyed (55°–60° C., for five minutes). The spores, if present, would resist such treatment. Consequently, if inoculations from this heated

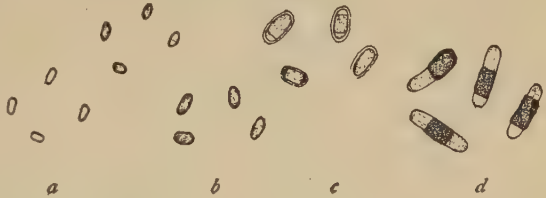


FIG. 43.—*Bacillus cohaerens*, Gottheil (*Bacillus bipolaris*, Burchard). Showing Bipolar Germination of Endospores. (After Burchard.)

culture into sterile media result in growth, one could conclude that the original culture produced heat-resistant spores. This method, of course, is not applicable to the thermophilic bacteria. In order to use this test with these organisms the culture should be boiled for 5 to 10 minutes.

Reproduction.—New bacterial cells are always formed by division of a preexisting cell. The progeny then grow and form two cells like the original one. With the rod bacteria, impending division is first noticeable with the appearance of a stricture in the cell wall which becomes more and more marked until the cell breaks at this point (Fig 44). For this reason bacteria are often spoken of as *fission fungi*. They resemble then certain other forms of life such as *Torulæ*, fission yeasts, and certain protozoa.

Sexual vs. Asexual Processes among the Bacteria.—Bacteriologists have generally stated that bacteria reproduced asexually, i.e., only one cell being involved. The characteristics of the progeny, then, are always identical with those of the parent cell. Recent observations lend support to the view that reproduction may not always be as simple as this.

It is probable that certain bacteria, if not all, at some stage in their life cycles show the union of two cells previous to reproduction. Sexual reproduction is just as probable among bacteria as among yeasts. In the latter group it has been convincingly demonstrated.

Rate of Reproduction.—One frequently hears the question, how often do bacterial cells divide? A few data are available but they should be interpreted only for the organisms studied and the conditions under which they were obtained. If this is not done, the answers to the question become very confusing. It is reasonable to expect that the temperature of incubation would have considerable influence as also the availability of food as well as the species of the organism. One investigator, Barber, isolated single cells of *Escherichia coli* (*Bacterium coli*) and placed them in hanging drops under the microscope where he could watch them for a considerable time in order to determine the actual rate

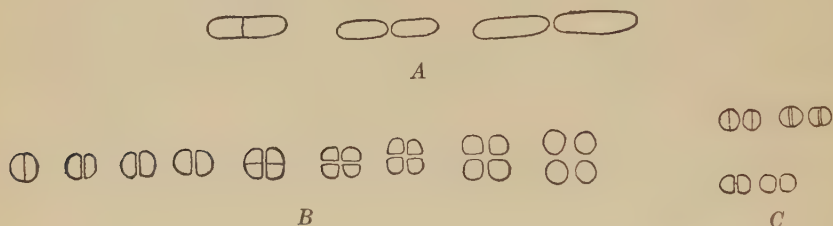


FIG. 44.—Diagrammatic Illustration of Cell Division. (After Migula.)

A. Rod-shaped Cells; B. Round Cells; C. Round Cells Showing Sarcina Grouping.

of division, or "generation time." He found that the generation time gradually decreased up to 40° C., after which it increased. The following results were secured with the above-mentioned organism.

20° C.	= 60 minutes
25° C.	= 41 minutes
30° C.	= 29.7 minutes
37° C.	= 17-21 minutes
40° C.	= 17+ minutes
42° C.	= 19-20 minutes
45° C.	= 30-34 minutes
50° C.	= no growth

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See also the list of reference texts in the appendix.

CHAPTER V

CHEMICAL COMPOSITION OF BACTERIA

The chemical composition of bacteria does not differ from that of other living organisms. The data depend on various conditions such as the age of the organism, species, and type of medium from which the cells are taken for analysis.

Elementary Composition.—This is quite like that of other cells. Such elements as nitrogen, carbon, hydrogen, sulfur, phosphorus, sodium, potassium, calcium with traces of other elements, are found. The elements which are present depend somewhat on the chemical composition of the medium in which the cells have been propagated. The storage of cells in physiological sodium chloride solution increases the sodium content. This would probably be true for most of the common soluble salts. Larson and his colleagues have stated that two types of salts exist in bacterial cells, free salts and fixed salts. The free salts are those which are easily removed by washing and consequently are transient salts in the cell. Their concentration is perhaps somewhat dependent on the salts which are in the medium about the cells. Fixed salts are those which are bound into the cell material. They are structural salts which cannot be removed from the cell by washing. Such a distinction explains some of the contradictory data on the salt content of bacterial cells as reported in the literature. Considerable work has been reported on the chemical constitution of bacteria. In Table II are quoted data on common bacteria by Nicolle and Allilaire. These data indicate that most of the bacterial cell is composed of water and that the rest of it is made up of materials such as fats, carbohydrates, etc., as are found in all cells irrespective of their origin.

The effect of culturing bacteria in various media is well shown by the data in Table III. This is strikingly shown by the data on the fat content. It will be seen that this is dependent on certain constituents in the media. One group of bacteria known as the "acid-fast group" includes microorganisms which contain larger amounts of fat than other bacteria. Table I reports data collected by Long and Campbell.

TABLE I

SHOWING THE LIPIN CONTENT OF VARIOUS ACID-FAST BACILLI. (AFTER LONG AND CAMPBELL.) · (AMER. REV. TUBERCULOSIS 6 (1922), 636-641)

	Total Lipin Per Cent of Dry Weight	Saponification Number	Per Cent of Total Lipins as Non- saponifiable
H37, human-type tubercle bacillus.....	22.7	131	77.1
B1, bovine-type tubercle bacillus.....	22.3	139	60.0
A1, avian-type tubercle bacillus.....	11.0	194	35.7
Leprosy bacillus, Duval.....	9.7	188	27.2
Turtle bacillus.....	36.3	191	28.3
Frog bacillus.....	37.1	195	33.7
Smegma bacillus.....	35.6	128	4.6
Dung bacillus.....	34.7	125	5.4
Grass bacillus.....	23.4	147	9.4
Grass III bacillus.....	30.3	109	4.5
Timothy bacillus.....	20.2		

Lipin Content of Other Microorganisms

Bacillus subtilis.....	4.4
Staphylococcus albus.....	2.8

TABLE II

SHOWING THE CHEMICAL COMPOSITION OF CERTAIN MICROORGANISMS. (AFTER NICOLLE AND ALLILAIRE, 1909)

Name of Organism*	Water, Per Cent	IN PER CENT DRY RESIDUE			Phos- phorus, Per Cent in Fat
		Nitrogen	Acetone Extract	Chloroform Extract	
Glanders.....	76.5	10.5	11.7	8.6	2.5
Chicken cholera.....	79.3	10.8	7.5	6.3	2.4
Cholera.....	73.4	9.8	8.7	6.8	2.4
Dysentery (Shiga).....	78.2	8.9	12.8	10.6	1.6
Proteus vulgaris.....	80.0	10.7	10.9	7.1	1.6
Typhoid.....	78.9	8.3	15.4	10.6	1.2
Anthrax.....	81.7	9.2	6.3	1.5	0.9
Pseudotuberculosis....	78.8	10.4	15.6	10.3	0.8
B. pneumoniae.....	85.5	10.4	15.4	10.3	0.8
B. coli.....	73.3	8.3	15.2	11.8	0.8
B. prodigiosus.....	78.0	10.5	9.0	6.6	0.5
B. psittacosis.....	78.0	9.5	11.1	7.0	0.5
B. diphtheriae.....	84.5		7.0	5.2	0.2
B. pyocyaneus.....	75.0	9.8	15.8	10.7	0.2

* The names are left as they were given by the French authors. In most cases they will be easily recognized by students.

TABLE III

SHOWING VARIATION IN CHEMICAL CONTENT OF *Escherichia coli* (Bact. *Coli*) IN
RELATION TO CHEMICAL COMPOSITION OF THE MEDIUM

(After Dawson, 1919)*

	MEDIUM							
	I	II	III	IV	V	VI	VII	VIII
	%	%	%	%	%	%	%	%
Water and volatile matter	74.84	72.5	60.25	75.01	74.9	60.69	60.32	79.55
Ash.....	4.8	2.7	2.5	4.5	4.5	7.83	7.69	2.1
Sulfur (total).....	0.06	0.0	0.0	0.1	0.09	0.0	0.0	0.14
Sulfur (loose).....	0.0	0.0	0.0	0.02	0.01	0.0	0.0	0.03
Phosphorus, P ₂ O ₅	4.24	3.48	2.38	2.89	3.30	1.69	1.83	0.92
Calcium, CaO.....	2.66	0.05	1.06	2.62	2.60	2.34	2.34	0.19
Nitrogen—total.....	2.843	3.02	6.22	2.405	2.115	4.327	5.005	5.027
Nitrogen—amino.....	0.771	0.891	2.970	0.724	0.691	1.696	1.650	3.012
Protein—Coagulation...	2.99	0.97	4.66	2.34	2.47	4.05	5.32	5.54
Protein—Acid precipitable	7.42	6.61	9.57	7.32	4.61	6.47	6.93	2.08
Protein—Alkali precipitable	5.60	7.44	2.80	6.05	5.33	6.63	6.80	4.22
Protein—Soluble.....	0.05	2.25	1.31	0.07	0.10	0.08	1.21	1.10
Protein—Insoluble.....	0.05	0.04	0.09	0.06	0.99	0.07	0.10	0.03
Residue—Insoluble.....	1.00	2.37	2.00	1.40	1.70	0.98	1.49	0.76
Fats.....	3.99	4.32	4.82	5.77	8.00	—	—	5.07
Carbohydrate.....	4.00	3.10	2.19	1.38	2.69	3.01	2.88	1.00
Cellulose-like-substance.	1.00	2.42	2.01	1.41	1.75	1.09	1.52	0.81

Composition of Media Used Above

- I. Peptone 1 per cent, meat extract 1 per cent. Neutral in reaction.
- II. Peptone 0.5 per cent, edestin 5 per cent. Neutral in reaction.
- III. Peptone 0.25 per cent; flour proteins 1 per cent. Alkaline in reaction.
- IV. Peptone 0.25 per cent; meat extract 1 per cent; glucose 1 per cent. Neutral in reaction.
- V. Peptone 0.25 per cent, meat extract 1 per cent; glucose 1 per cent; glycerol 1 per cent, neutral in reaction.
- VI. Peptone 0.25 per cent; butter soap 1 per cent.
- VII. Peptone 0.25 per cent; butter soap 1 per cent.
- VIII. Peptone none; potato juice from whole unskinned potato, freed from starch, 500 grams potato per liter of medium

* Dawson, Andrew Ignatius. 1919. Bacterial Variations Induced by Changes in the Composition of the Culture Media. Jour. Bact. 4, 133-148.

Chemical Composition of Different Parts of the Bacterial Cells.—The bacterial cell being made up of various organs would not be expected to have a homogeneous chemical composition. Each of the various organs has been examined for its own chemical make-up.

Cell Wall.—Much discussion has centered about the chemical constitution of the cell wall and the presence of cellulose and chitin therein. On the basis of the presence of cellulose, plant cells have been differentiated from animal cells. Cellulose has not been demonstrated in most bacterial cells; in a few, however, it or a substance chemically like it has been reported. Cellulose has been reported in *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae* and other microorganisms. Cellulose would add to the protective properties of the cell wall and in this respect is not an unexpected compound. The presence of cellulose would tend to emphasize the plant characteristics of the bacteria.

Another substance of significance in protective coverings is chitin. This substance is present in the shells of certain animals and has been reported in *Acetobacter xylinum*, *Bacillus anthracis* and others.

The presence of cellulose and chitin is of considerable phylogenetic significance. In analyzing the available data to decide whether bacteria are plants or animals it has been stated that the line of demarcation goes through the bacteria; part of the bacteria may possess more animal characteristics while part may possess more plant characteristics. The chemical evidence in this case may support this contention. Those bacteria which contain cellulose might be on the plant side while the chitin-containing microorganisms would fall on the animal side.

Capsule.—The capsule is a mucilaginous substance the chemical composition of which may also vary with the species, menstruum, etc. Some have reported a high content of mucin, compounds which possess both carbohydrate and protein characteristics.

Cytoplasm.—The cytoplasm is protein in nature but may contain substances of widely varying composition. Undecomposed food and the various products of its metabolism may be present. The type of protein is also specific for each species; this is relied upon for the identification of bacterial species by reactions which are discussed later in this book.

Spores.—The chemical constitution of spores probably does not differ from that of vegetative protoplasm. However, it seems quite generally agreed upon that spores contain less water than vegetative protoplasm. If it is true, there is a good basis for explaining the greater resistance of spores to heat and other destructive agents.

REFERENCES

- VAUGHAN, V. C. Protein Split Products in Relation to Immunity and Disease. Lea & Febiger, Philadelphia, 1913.
See also reference texts in the appendix.

CHAPTER VI

NOMENCLATURE AND CLASSIFICATION OF BACTERIA

In a former chapter were discussed some of the general principles of classification of living beings. Now we may take up the classification of bacteria. The difficulties of classification of bacteria are well indicated by the number of classifications which have been published and the storm of discussion which each new one stimulates. This need not worry the student who is beginning the study of microbiology, for the situation would be much worse without classifications. Such a student need be concerned only with the general principles and, perhaps, with several of the various classifications which have at times been proposed. The advanced student should give attention to this phase of the science. All sciences are supposed to make efforts to systematize knowledge. Chemists use periodic tables, and groups of closely related elements. They have such groups as the halogens, alkali metals, heavy metals, rare earths, etc. Those who work with living organisms have found it convenient to adopt the same practice of establishing groups composed of forms having salient characteristics in common.

Difficulties Encountered in the Classification of Bacteria.—Those who have attempted to classify bacteria have soon encountered difficulties the solution of which has been difficult to attain. Trouble arises possibly from the fact that the general principles applicable to higher groups of living organisms have been followed for classifying bacteria. Bacteria are such small organisms that their characteristics may not be conveniently determined from single cells. Great numbers of cells have to be used. Their rapid multiplication rate may also be responsible for the acquirement of new characteristics in a short period of time. The shape of bacterial cells seems to be influenced by certain conditions in the media. Life cycles have not been taken into account by those who have proposed classifications. These are some of the difficulties which have prevented the preparation of a satisfactory classification of bacteria. However, the situation is much brighter than some are willing to admit.

Early Classifications of Bacteria.—As soon as information accumulated about bacteria and different species began to be reported, bac-

terio logists began to arrange them in systematic manner to make classifications. Many of these early attempts are of historical interest only to-day since the necessity of pure cultures was not realized and methods of working which every bacteriologist uses to-day were not available. These classifications may be passed over in a book of this nature. They are reviewed in books on classification of bacteria, a number of which have been recently published.

Older Classifications.—We need mention but two of the older classifications since they have been rather widely used and have some merit. The first was prepared by Migula in 1895. This classification had wide popularity in America, more so than any other. The reason may not be apparent, although it may be due to the fact that Migula's classification was used by Chester in his "A Manual of Determinative Bacteriology" published in 1901. The second one is by Lehmann and Neumann. A revision of this one was published by Dr. Lehmann in 1927. The old one will be given here because it represents the older ideas in classification.

Migula's Classification of the Bacteria.—This classification has been widely used by American bacteriologists.

ORDER *EUBACTERIA*

Cells without nuclei, sulfur or bacteriopurpurin, colorless or but slightly colored, some forms chlorophyll-green.

FAMILY I. COCCACEAE

Cells when free spherical, in division sometimes elliptical. Division in one, two or three planes of space without previous elongation of the cell. Motility and endospore formation rare.

1. *Streptococcus*. Spherical cells dividing in one plane, often remaining attached to each other to form pairs or chains. Zooglea-like sheath or capsule common. Non-motile. No endospores.
2. *Micrococcus*. Division in two planes, sometimes forming bands of cells. Non-motile, endospores probably absent.
3. *Sarcina*. Division in three planes at right angles to each other, forming packets when the cells remain attached to each other. Non-motile. Endospore formation doubtful.
4. *Planococcus*. Division in two planes. Flagella present. Usually 1 to 2 in number. No endospores known.
5. *Planosarcina*. Division in three planes, pairs and tetrads usually seen rather than packets. Flagella present, usually one to each cell. No endospores.

FAMILY II. BACTERIACEAE

Cells cylindrical rods in free condition, dividing in a plane at right angles to their length. Short-celled forms may be distinguished from cocci by elongation before division. Cells may remain united, forming long or short threads. No sheath.

1. *Bacterium*. No flagella. Some form spores, others do not.
2. *Bacillus*. Peritrichic flagella. Spore formation common.
3. *Pseudomonas*. Polar flagella, 1 to 10 in number. Endospores in some forms.

FAMILY III. SPIRILLACEAE

Cells more or less spirally curved, division in one plane at right angles to long axis. Spore formation rare. Most forms motile, with polar flagella.

1. *Spirosoma*. Broad rigid cells, non-motile, free or in small zooglea groups.
2. *Microspira*. Cells usually comma- or sausage-shaped, motile, with one or rarely 2 to 3 polar flagella. Endospores not observed.
3. *Spirillum*. Long or short spirals, lophotrichic flagella, spores sometimes observed.
4. *Spirochaeta*. Slender spiral cells, generally long and flexible, showing serpentine and screw-like motions. Organs of motility unknown. Spores not observed.

FAMILY IV. CHLAMYDOBACTERIACEAE

Cylindrical cells arranged in threads with sheath. Reproduction by conidia which arise directly from the vegetative cells and develop into new threads without any resting stage.

1. *Chlamydothrix*. Unbranched filaments, segments often demonstrable by the use of reagents. Conidia non-motile.
2. *Crenothrix*. Unbranched filaments with differentiation between base and apex. Sheath thick and in iron waters often permeated with hydrate of iron. Cells cylindric or flat discoidal, conidia non-motile.
3. *Phragmidiothrix*. Very long threads with delicate sheath. Conidia non-motile.
4. *Sphaerotilus*. Cells in dichotomously branched threads, conidia motile.

ORDER THIOBACTERIA

Cells with no nucleus but with inclusions of sulfur, colorless or colored red, rose or violet with bacteriopurpurin. Never green.

This order includes the families Beggiatoaceae (genera *Thiothrix* and *Beggiatoa*), Rhodobacteriaceae (genera *Thiocystis*), *Thiocapsa*, *Thiosarcina*, *Lamproystitis*, *Thiopedia*, *Amoebabacter*, *Thiothece*, *Thiodictyon*, *Thiopolycoccus chromatium*, *Rhabdochromatium* and *Thiospirillum*, into the classification of which we need not enter here.

Lehmann and Neumann's Classification of the Bacteria.—Another classification which has enjoyed rather widespread use was prepared by Lehmann¹ and Neumann.

I. COCCACEAE

1. *Streptococcus*. Dividing in one plane.
2. *Sarcina*. Dividing in three planes.
3. *Micrococcus*. Irregular division, including all but clearly marked chains and packets.

¹ Dr. Lehmann published a more elaborate classification of bacteria in 1927. The earlier one is given here since it represents in a better way the principles followed a few years ago.

II. BACTERIACEAE

1. *Bacterium*. Without endogenous spores, rods usually $0.8-0.1\mu$ in diameter.
2. *Bacillus*. With endogenous spores, rods often more than 1.0μ in diameter.

III. SPIRILLACEAE

1. *Vibrio*. Short, rigid, slightly curved cells, with 1 to 2 polar flagella.
2. *Spirillum*. Long rigid spiral cells, with lophotrichic flagella.
3. *Spirochaeta*. Long flexible spiral cells, flagella unknown motility accomplished by an undulating membrane.

SUPPLEMENTARY GROUP

ACTINOMYCETES

Thread-like cells with true branching, sometimes even a richly branching mycelium. Young cultures often show only normal unbranched bacterial cells. Many forms show a tendency to the formation of irregular clubbed cells.

1. *Corynebacterium*. Slender, often slightly curved rods, often with a tendency to club formation, branching rare in young cultures and often difficult to find even in old ones. Neither flagella nor spores known. With weak stains show barred staining. Not acid-fast.
2. *Mycobacterium*. Same as above except for the fact that the cells are acid-fast and take ordinary stains with difficulty, and that club forms are very rare outside the body.
3. *Actinomyces*. Long, mycelial threads, showing true branching. Spores formed by fragmentation and cross-division of filaments. Many forms produce a mold-like aerial mycelium. Not acid-fast. Motility sometimes present. Most forms produce a moldy odor.

A comparison of these classifications brings out some differences. It will be seen that Migula separates a *Bacillus* from a *Bacterium* on the basis of motility, the former being motile and the latter non-motile. Lehmann and Neumann separate these genera on the basis of spore formation, *Bacterium* forming no spores and *Bacillus* forming spores. Both of these classifications are morphological, based on shape and structure. The use of different classifications thus results in slightly different names for the same organism. These names frequently differ only in the generic portion and little confusion results.

Nomenclature of Bacteria.—This subject should be presented to students who are beginning the study of bacteriology because they will become the bacteriologists of the future. Fischer stated that the factor to which the progress of bacteriology is mainly due has also been a hindrance, for we have bacteriologists, agriculturists, brewers, botanists, etc., all vying with one another to report new species. No one should report new species without first a continued intensive study of the supposed new organism, without a careful com-

parison with all similar bacteria which have been reported by others, and finally, without essential differences between the new form and those already described. Reporting of new species should be regarded as a more serious matter than it is by some bacteriologists. As an example of the confusion which exists, the various names for *Clostridium welchii* may be given: *Bacillus aerogenes capsulatus*, *Bacillus phlegmonis emphysematosae*, *Bacillus perfringens*, *Bacillus enteritidis sporogenes*. Some confusion must be expected but the present situation may be prevented to some extent in the future if students are introduced to the situation as it exists to-day.

In order to settle controversial questions as satisfactorily as possible, scientists have resorted to international congresses at which representatives from all nations could express their opinions. These have been very useful for the botanists and biologists, but have not been employed by bacteriologists for discussing such questions as classification and nomenclature. These congresses have not entirely eliminated differences of opinion but have resulted in some improvement and uniformity.

The binomial system proposed by Linnaeus has been accepted by scientists as the best one to follow for naming living organisms. According to this system, each living creature has a name composed of two parts—a generic name indicating the genus and a specific name for the species. The generic name is always capitalized while the specific name generally is not. Sometimes the specific name is capitalized when it has been derived from the name of a town or individual, but many authors consistently use the lower-case letter. It is also proper to append to the name of the organism the name of the discoverer (without punctuation) with the date (set off by a comma), of the earliest report in the literature which contains the name, as *Bacillus anthracis* Pollender, 1849. Often one sees the name of the author in parentheses. This means that he proposed the specific name but placed it in another genus than that in which it is now placed; the name of the individual responsible for placing the organism in its present genus is written after the name of the original author of the species. Thus, *Micrococcus luteus* (Schroeter, 1872) Cohn, 1872 means that Schroeter gave the organism its specific name but placed it in another genus (*Bacteridium luteum*). Cohn, however, placed it in the genus in which it now appears.

The species name need not necessarily be appropriate to the organism. Names are only handles which enable bacteriologists conveniently to discuss bacteria with others. Names represent groups of characteristics. They have the same function for bacteria that our names have for us. Some bacterial names are decidedly inappropriate just as in the case with names of human beings. However, this does not

need to affect the validity of the name if it does not violate any of the well-established rules of nomenclature. Bearing this in mind, it is more convenient, however, when the species name gives some information about the greatest importance of the organism, if it has such. The name *Eberthella typhi* (*Bacterium typhosum*) is perhaps a good name since it suggests a relation to typhoid fever.

Some bacteria are best known, not by their scientific names, but by names derived from some outstanding property. For instance, we more frequently hear the expression "tubercle bacillus" for *Mycobacterium tuberculosis*, "hay bacillus" for *Bacillus subtilis*, "potato bacillus" for *Bacillus mesentericus*, etc. Some organisms, such as the "clam bacillus" have not been identified and classified and are only known by this so-called pseudoscientific name.

The Descriptive Chart and Index Number.—About thirty-five years ago a few bacteriologists in America believed that, if bacteria were studied according to a definite outline, more uniformity would exist and there might be less possibility of an organism appearing in the literature under different names. This effort finally culminated in the "Descriptive Chart" of the Society of American Bacteriologists, a copy of the latest edition of which is inserted in this book. Examinations of it will reveal that the salient characteristics of an unknown organism are listed.

One feature of the Descriptive Chart is the Index Number formerly called the Group Number. This Index Number is the result of an attempt to give a numerical expression to the major characteristics of bacteria. It does not mean that this index number will or should replace names. It is convenient, however, for comparing a large number of strains of one organism or the flora of a certain material. By means of the index number the organisms may be arranged into groups. For instance, Hucker in an intensive study of the spherical bacteria used the index number with very slight modifications for recording his observations.

Characteristics Used for Classifying Bacteria.—What characteristics are used for classifying bacteria? Examination of the classifications reproduced in this chapter will reveal that shape of the cell (morphology) was one of the first characteristics to be used. With this three large groups were made. Morphological characteristics were also used for dividing these larger groups. Space relationship has been used for subdividing the round cells, formation of endospores or the presence or absence of organs of locomotion for the cylindrical cells, and the degree of the bend or twist for the members of the third group. The use of such characteristics gives what are called morphological classifications.

Endorsed by SOCIETY OF AMERICAN BACTERIOLOGISTS
at the annual meeting Dec. 30, 1920

DESCRIPTIVE CHART

Prepared by H. J. CONN
K. N. ATKINS
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J. F. NORTON
G. E. HARTON
Committee on
bacteriological
technic

Name of organism.....Source.....Date of isolation.....Studied by.....Culture No.....
INVIGORATION OF CULTURE: Date.....Medium used.....Temperature.....Number of transfers.....Length of each incubation... days. Series No.....

MORPHOLOGY

NOTE—Underscore required terms.	SKETCHES
VEGETATIVE CELLS, Medium used..... temp.....age.....days. Form, spheres, short rods, long rods, filaments, commas, short spirals, long spirals, curved. Arrangement, single, pairs, chains, fours, clusters, cubical packets. Limits of Size.....Size of Majority..... Ends, rounded, truncate, concave.	
CAPSULES, present on..... How stained.....	
SPORANGIA, present, absent. Medium used..... temp.....age.....days. Form, elliptical, short rods, spindled, clavate, drum- sticks. Limits of Size.....Size of Majority.....	
ENDOSPORES, present, absent. Location of Endospores, central, polar. Form, spherical, elliptical, elongated. Limits of Size..... Size of Majority..... Wall, thick, thin. Sporangium wall, adherent, not adherent.	
MOTILITY In broth.....On agar.....	
FLAGELLA, No.....Attachment, polar, bipolar, peritrichiate. How stained.....	
IRREGULAR FORMS, Present on.....in.....days at.....°C. Form, spindled, cuneate, filamentous, branched, or.....	
STAINING REACTIONS. Gram.....Acid fast..... Special stains.....	

CULTURAL CHARACTERISTICS

Underscore required terms	SKETCHES	Underscore required terms
Agar Stroke Incubation Temperature°C Aged		Nutrient Broth Temperature°C Aged
Gelatin Stab Temperature°C Aged		Medium (liquid) Temperature°C Aged
Medium (solid) Temperature°C Aged		Medium Temperature°C Aged

Index No.*.....

BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below:

PRIMARY CHARACTERISTICS	Microscopic Features	Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous
		Spores: 1, central; 2, polar; 3, absent
		Flagella: 1, peritrichic; 2, polar; 3, absent
Miscellaneous Biochemical Reactions		Gram stain: 1, positive; 2, negative
		Pathogenicity, etc.: 1, for man; 2, for animals; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic
		Relation to oxygen: 1, strict aerobe; 2, facultative anaerobe; 3, strict anaerobe
Carbohydrate Reactions		Gelatin liquefaction: 1, positive; 2, negative
		In nitrate media: 1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas
		Chromogenesis: 1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none
Vegetative Cells		Diastatic action: 1, positive; 2, negative
		From dextrose: 1, acid and gas; 2, acid without gas; 3, no acid
		From lactose: 1, acid and gas; 2, acid without gas; 3, no acid
Spores		From sucrose: 1, acid and gas; 2, acid without gas; 3, no acid
		Diameter: 1, under 0.5μ; 2, between 0.5μ and 1μ; 3, over 1μ
		Length: 1, less than 2 diameters; 2 more than 2 diameters
Cultural Features		Chains (4 or more cells): 1, present; 2, absent
		Capsules: 1, present; 2, absent
		Shape: 1, round; 2, oval to cylindrical
Agar Stroke		Diameter: 1, less than diameter of rod; 2, greater than diameter of rod
		Abundance: 1, abundant; 2, moderate; 3, slight; 4, absent
		Lustre: 1, glistening; 2, dull
Milk		Surface: 1, smooth; 2, contoured; 3, rugose
		Agar colonies: 1, punctiform; 2, round (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled
		Gelatin colonies: 1, punctiform; 2, round (over 1 mm.); 3, irregular; 4, filamentous
		Acid: 1, sufficient for curdling; 2, insufficient for curdling; 3, no acid
		Rennet curd: 1, present; 2, absent
		Peptonization: 1, present; 2, absent

* Recording the "Index Number" here is optional; but its use will be found convenient if the charts are to be filed according to the salient characteristics of the organisms. The Index Number consists of the first thirteen figures from the margin (primary characteristics) copied down in the order of their occurrence in the margin, placing a dash wherever a heavy rule occurs in the margin. Thus, B coli belongs to the group 5312-41220-1111.

	Growth, <i>slow, rapid.</i>	SKETCHES
Age	Form, <i>punctiform, circular, irregular, mycelioid, filamentous.</i>	
Color	Elevation, <i>flat, raised, convex, pulvinate, crateriform (liquefying).</i>	
Temperature	Edge, <i>entire, undulate, lobate, erose, filamentous, floccose, curled.</i>	
Age	Liquefaction, <i>cup, saucer, spreading.</i>	
	Internal structure, <i>amorphous, finely-, coarsely-granular, filamentous, curled, concentric.</i>	

		FERMENTATION					
Optimum	°C.						Temperature.....°C.
Maximum	rate; better						
Minimum							
		Medium.....	DEXTROSE	LACTOSE	SACCHAROSE	GLYCERIN	
		containing.....					
							
							
		 and:					
Nutrient		Gas					
Nutrient		First appearance of acid.....					
Potato	days.....	First appearance of alkali.....					
	days.....	Reaction after..... days					
	days.....	Reaction after..... days					
Medium		Max. H-ion Conc.					
Indol,							
	°C.						
	days.....						
PR	days.....						
Medium	°C.						
	days.....						
H ₂ S, a	days.....						

IOGENICITY)

Physiological characters are also used. The physiological or functional characteristics are those which result from the metabolic activities of the organism. These, then, include the action of the organism on carbohydrates, proteins fats, etc. They generally require the use of chemical tests for studying them and many of these are carried out by the student in the laboratory. Physiological characters have been used for establishing large groups of bacteria having some salient property in common. The "lactic acid" bacteria make up a physiological group since bacteriologists place in this group those bacteria which form appreciably large amounts of lactic acid. This is not a safe method of final grouping because the property used for making the group may be exhibited by other bacteria only to a lesser degree. This method also brings together forms which are quite unlike with respect to other characters which are well established in taxonomic work. Probably all forms of life produce a little lactic acid and might, therefore, be placed in the "lactic acid group." Other groups created by the utilization of physiological characteristics are chromogenic, zymogenic, acetic acid, etc. The pathogenic group is one of marked interest. It is based on the ability of its members to cause infections in animals and plants. The pathogenic group is divided into sub-groups mainly on the basis of certain characteristics of the members.

Classification Proposed by the Committee on the Characterization and Classification of Bacterial Types of the Society of American Bacteriologists.—Realizing the inadequacies of the older classifications, the Society of American Bacteriologists appointed a committee to study the situation. The work of this committee culminated in two reports the last of which was published in the *Journal of Bacteriology* for 1920. This classification was later modified by Bergey in his "Manual of Determinative Bacteriology." There seems to be some difference of opinion over the designation of the classification prepared by the Society of American Bacteriologists' Committee on the Characterization and Classification of Bacterial Types whether it shall be called the Society of American Bacteriologists' classification or the Committee's classification. The latter seems to be favored more by those who are familiar with the situation.²

² The author has had considerable doubt about what classifications should be presented in this chapter and just how far the problems of classification should be taken up. He has, however, after discussion with students of the question, given two of the older and two of the new ones. The latter are those prepared by the Committee of the Society of American Bacteriologists and Bergey's modification of it. The latter have been criticized destructively—but what classification has not? Those who prepared these classifications gave them much study, probably much more than the critics. These newer classifications prepared by committees are cer-

THE CLASS OF SCHIZOMYCETES

Minute, one-celled, chlorophyll-free, colorless rarely violet-red or green-colored plants, which typically multiply by dividing in one, two or three directions of space. The cells thus formed are usually spherical, cylindrical, comma-shaped, spiral or filamentous, and are often united into filamentous, flat, or cubical aggregates. Filamentous species often surrounded by a common sheath. The cell plasma generally homogeneous without a morphologically differentiated nucleus. Reproduction by simple fission. In many species resting bodies are produced, either endospores or gonidia. Cells may be motile by means of flagella.

A. ORDER MYXOBACTERIALES

Cells united during vegetative stage into a pseudoplasmodium which passes over a highly developed cyst-producing resting stage.

B. ORDER THIOBACTERIALES

Cells free or united in elongated filaments. Typically water forms, not cultivable in ordinary media. Life energy derived mainly from oxidative processes. Cells typically containing either granules or free sulfur or bacteriopurpurin or both, usually growing best in the presence of hydrogen sulfide.

C. ORDER CHLAMYDOBACTERIALES

Cells normally united in elongated filaments often showing false but never true branching. Typically water forms. Sulfur and bacteriopurpurin are absent. Iron often present and usually a well-marked sheath.

D. ORDER ACTINOMYCETALES

Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings and clubbed or irregular shapes. No pseudoplasmodium. No deposits of free sulfur or iron. No bacteriopurpurin. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Some species are parasitic in animals or plants. Not water forms. Complex proteins frequently required. As a rule strongly aerobic (except for some species of *Actinomyces* and the genera *Fusiformia* and *Leptotrichia*) and oxidative. Growth on culture media often slow; some genera show mold-like colonies.

FAMILY I. ACTINOMYCETACEAE

Filamentous forms often branched and sometimes forming mycelia. Conidia sometimes present. Some species parasitic.

tainly an improvement over the older ones which, with few exceptions, were prepared by one individual. Names of bacteria according to the newer ideas of classification are appearing with greater frequency in bacteriological literature. The present confusion and controversy in the fields of nomenclature and classification may be due to the fact that these subjects have not been faced squarely by teachers. Why wait for the perfect classification instead of stating the actual situation as it exists?

GENUS 1. ACTINOBACILLUS

Filament formation, resembling streptobacilli. In lesions no mycelium formed, but at peripheries finger-shaped branched cells are visible. Gram-negative. Not acid-fast.

Type species, *Act. Lignieresii* Brumpt.

GENUS 2. LEPTOTRICHIA

Synonyms: *Leptothrix* Robin, 1847, not *Leptothrix* Keutzing, 1843; not *Leptothrix* Zopf, 1885; *Rasmussenia* Trevisan, 1889.

Thick, long, straight or curved threads, unbranched, frequently clubbed at one end and tapering to the other. Gram-positive when young. Threads fragment into short, thick rods. Anaerobic or facultative. Non-motile. Filaments sometimes granular. No aerial hyphae or conidia. Parasites or facultative parasites.

The type species is *Leptotrichia buccalis* (Robin, 1847) Trevisan.

GENUS 3. ACTINOMYCES HARZ, 1877, p. 125

Synonyms: *Streptothrix* Cohn, 1875, not *Streptothrix* Corda, 1839; *Discomyces* Rivolta, 1879; *Norcardia*, Trevisan, 1889; *Micromyces* Gruber, 1891, not *Micromyces* Dangeard, 1888; *Oospora* Sauvageau and Radais, 1892; not *Oospora* Wallroth, 1833; *Thermoactinomyces* Tsilinsky, 1899; *Cohniastreptothrix* Pinoy, 1913.

Organism growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Sometimes parasitic, with clubbed ends of radiating threads conspicuous in lesions in animal body. Some species are micro-aerophilic or anaerobic. Non-motile.

The type species is *Actinomyces bovis* Harz.

GENUS 4. ERYSIPELOTHRIX

Rod-shaped organisms with a tendency to the formation of long filaments which may show branching. The filaments may also thicken and show characteristic granules. No spores. Non-motile. Gram-positive. Do not produce acid. Micro-aerophilic. Usually parasitic.

The type species is *Erysipelothrix rhusiopathiae* (*Bacillus rhusiopathiae suis* Kitt, 1893; *Mycobacterium rhusiopathiae* Chester, 1901; *Erysipelothrix porci* Rosenbach, 1909), the causal organism of swine erysipelas.

FAMILY II. MYCOBACTERIACEAE

Parasitic forms. Rod-shaped, frequently irregular in form, but rarely filamentous and with only slight and occasional branching. Often stain unevenly (showing variations in staining reaction within the cell). No conidia.

GENUS 1. MYCOBACTERIUM

Synonyms: *Coccothrix* Lutz, 1886; *Sclerothrix* Metschnikoff, 1888, not *Sclerothrix* Kuetzing, 1849; *Mycomonas* Jensen, 1909.

Slender rods which are stained with difficulty, but when once stained are acid-fast. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even-branched cells. Non-motile Gram-positive. No endospores. Growth on media slow. Aerobic. Several species pathogenic to animals.

The type species is *Mycobacterium tuberculosis* (Koch, 1882) Lehmann and Neumann.

GENUS 2. CORYNEBACTERIUM

Synonyms: *Corynemonas* Jensen, 1909; *Corynethrix* Bongert, 1901.

Slender, often slightly curved, rods with tendency to club and pointed forms, branching cells reported in old cultures. Barred uneven staining. Liquefied. Do not ferment carbohydrates. Growth on potato characteristically honey-like.

Type species, *Pfeifferella mallei* (Loeffler, 1886) Buchanan (the glanders bacillus).

The real lines of demarcation between the genera *Actinobacillus*, *Erysipelothrix*, *Fusiformis* and *Pfeifferella* and their relations to *Actinomyces* on the one hand and to *Mycobacterium* on the other seem very obscure and the above arrangement can be considered as only tentative.

E. ORDER EUBACTERIALES

The order Eubacteriales includes the forms usually termed the true bacteria, that is, those forms which are considered least differentiated and least specialized. The cell metabolism is not primarily bound up with hydrogen sulfide or other sulfur compounds, the cells in consequence containing neither sulfur granules nor bacteriopurpurin. The cells apparently do not possess a well-organized or well-differentiated nucleus. These organisms are usually minute and spherical, rod-shaped or spiral, in most genera not producing true filaments; and rarely branching. The cells may occur singly, in chains or other groupings. They may be motile by means of flagella, or non-motile; but are never notably flexuous. Cell multiplication occurs always by transverse, never by longitudinal fission. Some genera produce endospores, particularly the rod-shaped types. Conidia not observed. Chlorophyll is absent, though the cells may be pigmented. The cells may be united into gelatinous masses, but never form motile pseudo-plasmodia nor develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the *Myxobacteriales*.

FAMILY I. NITROBACTERIACEAE

Organisms usually rod-shaped (sometimes nearly spherical in *Nitrosomonas* and possibly in *Azotobacter*). Cells motile or non-motile. Branched involution forms in *Rhizobium* and *Acetobacter*. Endospores never formed. Obligate aerobes, capable of securing growth energy by the direct oxidation of carbon, hydrogen or nitrogen or of simple compounds of these. Non-parasitic (except in genus *Rhizobium*)—usually water or earth forms.

TRIBE 1. NITROBACTEREAE

Organisms deriving their life energy from oxidation of simple compounds of carbon and nitrogen (or of alcohol).

GENUS 1. HYDROGENOMONAS

Monotrichic short rods capable of growing in the absence of organic matter, and securing growth energy by the oxidation of hydrogen (forming water). Kaserer, (1905) who first described the organism, states that his species will also grow well on a variety of organic substances.

The type species is *Hydrogenomonas pantotropha* (Kaserer, 1906), Orla-Jensen, Nikleuski (1910), described two additional species, *H. vitrea* and *H. flava*.

GENUS 2. METHANOMONAS

Monotrichic short rods capable of growing in the absence of organic matter and securing growth energy by the oxidation of methane (forming carbon dioxide and water).

The type species is *Methanomonas methanica* (Söhngen, 1906), Orla-Jensen.

GENUS 3. CARBOXYDOMONAS

Autotrophic rod-shaped cells capable of securing growth energy by the oxidation of carbon monoxide (forming carbon dioxide).

The type species, *Carboxydomonas oligocarbophila* (Beijerinck and van Delden, 1903), Orla-Jensen, is described as non-motile.

GENUS 4. ACETOBACTER

Synonyms: *Mycoderma* Persoon, 1822; *Ulvina* Keutzing, 1837; *Umbina* Naegeli, 1849; *Bacteriopsis?* Trevisan, 1885; *Gliacoccus* Maggi, 1886; *Acetimonas* Jensen, 1909.

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually on the surface of alcoholic solutions as obligate aerobes, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells may occur as involution forms.

The type species is *Acetobacter aceti* (Thomson, 1852), Committee.

GENUS 5. NITROSOMONAS

Includes *Nitrosococcus* Winogradsky, 1892.

Cells rod-shaped or spherical. Motile or non-motile; if motile, with polar flagella. Capable of securing growth energy by the oxidation of ammonia to nitrites. Growth on media containing organic substances scanty or absent.

The type species is *Nitrosomonas europaea* Winogradsky.

GENUS 5. NITROBACTER WINOGRADSKY

Synonym: *Nitrosobacterium?* Rullmann, 1897.

Cells rod-shaped, non-motile, not growing readily on organic media or in the presence of ammonia. Cells capable of securing growth energy by the oxidation of nitrites to nitrates.

The type species is *Nitrobacter Winogradskyi*, Committee, 1917a, p. 552.

TRIBE 2. AZOTOBACTEREAE

Nitrogen-fixing organisms.

GENUS 7. AZOTOBACTER

Synonyms: *Parachromatium* Beijerinck, 1903; *Azotomonas*, Jensen, 1909.

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates. Motile or non-motile; when motile, with tuft of polar flagella. Obligate aerobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen.

The type species is *Azotobacter chroococcum* Beijerinck.

GENUS 8. RHIZOBIUM

Synonyms: *Phytomyza* Schroeter, 1886; *Cladochytrium* Vuillemin, 1888; *Rhizobacterium* Kirchner, 1895; *Pseudorhizobium* Hartleb, 1900; *Rhizomonas* Jensen, 1909.

Comment. *Phytomyza* Schroeter has priority over *Rhizobium*, but because of the confusion which would arise from the substitution of the older correct name for the better-known term *Rhizobium*, the committee recommends the adoption of the latter.

Minute rods, motile when young. Involution forms abundant and characteristic when grown under suitable conditions. Obligate aerobes, capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates in the absence of compounds of nitrogen. Produce nodules upon the roots of leguminous plants.

The type species is *Rhizobium leguminosarum* Frank.

FAMILY II. PSEUDOMONADACEAE

Rod-shaped, short, usually motile by means of polar flagella or rarely non-motile. Aerobic and facultative. Frequently gelatin liquefiers and active ammonifiers. No endospores. Gram stain variable, though usually negative. Fermentation of carbohydrates as a rule not active. Frequently produce a water-soluble pigment which diffuses through the medium as green, blue, purple, brown, etc. In some cases a non-diffusible yellow pigment is formed. Many yellow species are plant parasites.

GENUS 1. PSEUDOMONAS

Synonyms: *Bacterium* Ehrenberg emended Cohn, 1872; *Bactrillum* Fischer, 1895; *Arthrobactrinium* Fischer, 1895; *Arthrobactrillum* Fischer, 1895; *Eupseudomonas* Migula, 1895; *Bactrinus* Kendall, 1902; *Bactrillius* Kendall, 1902; *Bacterium* Ehrenberg emended E. F. Smith, 1905; *Denitromonas* Jensen, 1909; *Liquidomonas* Jensen, 1909.

Characters, those of family.

Type species, *Ps. aeruginosa* (Schroeter) Frost

FAMILY III. SPIRILLACEAE

Cells elongate, more or less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous. Usually without endospores. As a rule motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

GENUS 1. VIBRIO

Synonyms: *Pacinia* Trevisan, 1885; *Microspira* Schroeter, 1886; *Pseudospira* De Toni and Trevisan, 1889; *Liquidovibrio* Jensen, 1909; *Solidovibrio* Jensen, 1909; *Photobacterium*? Beijerinck, 1889.

Cells short, bent rods, rigid, single or united into spirals. Motile by means of a single (rarely two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aerobic and anaerobic. No endospores. Usually gram-negative. Water forms, a few parasites.

The type species is *Vibrio comma* (Koch, 1884), Schroeter, 1886.

GENUS 2. SPIRILLUM

Synonyms: *Spirobacillus*? Metschnikoff, 1889; *Spirosoma* Migula, 1894; *Sporospirillum*? Jensen, 1909.

Cells, rigid rods of various thicknesses, length, and pitch of the spiral, forming

either long screws or portions of a turn. Usually motile by means of a tuft of polar flagella (5 to 20) which are mostly half circular, rarely wavy-bent. These flagella occur on one or both poles: their number varies greatly and is difficult to determine; since in stained preparations several are often united into a common strand. Endospore formation has been reported in some species. Habitat: water or putrid infusions.

Type species *Spirillum undula* (Mueller, 1786) Ehrenberg.

FAMILY IV. COCCACEAE

Synonyms: *Sphaerobacteria* Cohn, 1872; *Coccaceen* Zopf, 1884; *Coccogenae* Trevisan, 1885; *Coccacei* Schroeter, 1886; *Coccobacteria* Schroeter, 1886; *Sphaerobacteries* Maggi, 1886; *Kokkaceen* Hueppe, 1886; *Coccacees* Mace, 1897.

Cells in their free conditions, spherical; during division somewhat elliptical. Division in one, two or three planes. If the cells remain in contact after division they are frequently flattened in the plane of division, and form chains, packets or irregular masses. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates. Pigment often produced. Motility rare. Endospores absent.

TRIBE A. NEISSEREA

Strict parasites, failing to grow or growing very poorly on artificial media. Cells normally in pairs. Gram-negative. Growth fairly abundant on serum media.

GENUS 1. NEISSERIA

Synonyms: *Diplococcus* Weichselbaum, 1886 in part; *Gonococcus?* Neisser? 1879; *Merismopedia* Zopf, 1885; *Merismopedia* Meyen, 1839.

Characters, those of tribe.

Type species, *N. gonorrhoea* Trevisan.

TRIBE B. STREPTOCOCCAE

Parasites (thriving only or best on or in the animal body) except genus *Leuconostoc*. Grow well under anaerobic conditions. Many forms grow with difficulty on serum-free media, none very abundantly. Planes of fission usually parallel, producing pairs or short or long chains, never packets. Generally stain by Gram. Produce acid but no gas in glucose and generally in lactose broth. Pigment, if any, white or orange.

GENUS 2. DIPLOCOCCUS

Synonyms: *Klebsiella* Trevisan, 1885, in part; *Hyalococcus* Schroeter, 1886; *Pseudodiplococcus* Bonome, 1888; *Pneumococcus?* Schmidlechner, 1905.

Parasites, growing poorly, or not at all, on artificial media. Cells usually in pairs of somewhat elongated cells, often capsulated, sometimes in chains. Gram-positive. Fermentative powers high, most strains forming acid in glucose, lactose, sucrose and inulin.

Type species, *D. pneumoniae* Weichselbaum.

GENUS 3. LEUCONOSTOC

Synonyms: *Ascococcus* Cienkowski, 1878; not *Ascococcus* Cohn, 1887; *Leucocystis?* Schroeter, 1886.

Saprophytes, usually growing in cane-sugar solutions. Cells in chains or pairs, united in large zooglycal masses. Some types at least gram-negative.

Type species, *L. mesenteroides* (Cienkowski) Van Tieghem.

GENUS 4. STREPTOCOCCUS

Synonyms: *Sphaerococcus* Marpamann, 1885, not *Sphaerococcus* Agardh, 1821; *Arthrostreptokokkus* Hueppe, 1886; *Perroncilloa* Trevisan, 1889; *Babesia?* Trevisan, 1889; *Schuetzia* Trevisan, 1889; *Lactococcus* Beijerinck, 1901; *Hypnococcus* Bettencourt et al. 1904. *Myxokokkus* Gonnermann, 1907, not *Myxococcus* Thaxter, 1892; *Melococcus?* Amiradzibi, 1907; *Diplostreptococcus* Lingesheim, 1912.

Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). Generally stain by Gram. Capsules rarely present, no zooglycal masses. On agar streak, effused translucent growth, often with isolated colonies. In stab culture, little surface growth. Many sugars fermented with formation of large amount of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates.

Type species is *Streptococcus pyogenes* Rosenbach.

GENUS 5. STAPHYLOCOCCUS

Synonyms: *Micrococcus* Cohn, 1872 em. Migula, 1894; *Botryomyces* Bollinger, 1888; *Batryococcus* Kitt, 1888, not *Botryococcus* Kuetzing, 1849; *Galactococcus* Guillebeau; *Bollingera* Trevisan, 1889; *Gaffkya* Trevisan, 1885; *Pyococcus* Ludwig, 1892; *Carphococcus* Hohl, 1902; *Albococcus* Winslow and Rogers, 1906; *Aurococcus* Winslow and Rogers, 1906; *Indolococcus* Jensen, 1909; *Liquidococcus* Jensen, 1909; *Peptonococcus* Jensen, 1909; *Enterococcus?* (Thiercelin) Rougentzoff, 1914.

Parasites. Cells in groups and short chains, very rarely in packets. Generally stain by Gram. On agar streak good growth, of white or orange color. Glucose, maltose, sucrose and often lactose, fermented with formation of moderate amount of acid. Gelatin often liquefied very actively.

TRIBE C. MICROCOCCAE

Facultative parasites or saprophytes. Thrive best under aerobic conditions. Grow well on artificial media, producing abundant surface growths. Planes of fission often at right angles; cell aggregates in groups, packets or zooglycal masses. Generally decolorize by Gram. Pigment yellow or red.

GENUS 6. MICROCOCCUS

Synonyms: *Microsphaera* Cohn, 1872, not *Microsphaera* Leveille, 1851; *Ascococcus* Cohn, 1875; *Pediococcus* Baleke, 1884; *Merista* Van Tieghem, 1884, not *Merista* (Banks and Soland), Cunningham, 1839; *Planococcus* Migula, 1894; *Urococcus* Miquel, 1879; not *Urococcus* Kuetzing, 1849; *Carphococcus* Hohl, 1902; *Pedioplana* Wolff, 1907; *Tetradiplococcus?* Bartoszewicz and Schwarzwasser, 1906; *Solidococcus* Jensen, 1909; *Planomerista* Vuillemin, 1913.

Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or packets). Generally decolorize by Gram. Growth on agar abundant, with formation of yellow pigment. Glucose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied, but not rapidly.

The type species is *Micrococcus luteus* (Schroeter), 1872b, Cohn.

GENUS 7. *SARCINA*

Synonyms: *Urosarcina* Miquel, 1879; *Planosarcina* Migula, 1894; *Pseudo-sarcina*? Maze, 1903; *Tetradiplococcus*? Bartoszewicz and Schwarzwasser, 1908; *Lactosarcina* Beijerinck, 1908; *Sporosarcina*? Jensen, 1909.

Sarcina differs from *Micrococcus* solely in the fact that cell division occurs under favorable conditions in three planes, forming regular packets.

The type species is *Sarcina ventriculi* Goodsir.

GENUS 8. *RHODOCOCCLUS*

Synonyms: Not *Rhodococcus* Molisch, 1907.

Saprophytes. Cells in groups or regular packets. Generally decolorize by Gram. Growth on agar abundant with formation of red pigment. Glucose broth slightly acid, lactose broth neutral. Gelatin rarely liquefied. Nitrates generally reduced.

Type species, *Rhodococcus rhodochrous* Zopf.

FAMILY V. BACTERIACEAE

Rod-shaped cells without endospores. Usually Gram-negative. Flagella when present peritrichic. Metabolism complex, amino-acids being utilized, and generally carbohydrates.

TRIBE 1. CHROMOBACTEREAE

Water bacteria producing a red or violet pigment.

GENUS 1. *ERYTHROBACILLUS*

Synonyms: *Zoagalactina* Sette, 1824; *Serratia* Bizio, 1825; *Bacillus*, in part, of many authors.

Small aerobic bacteria, producing a red or pink pigment, usually a lipochrome. Gram stain variable. It is possible that related yellow and orange chromogens should be included here as well.

Type species, *Erythrobacillus prodigiosus* (Ehrenberg) Committee.

GENUS 2. *CHROMOBACTERIUM*

Synonyms: The name was spelled *Cromobacterium* by Bergonzini and corrected by Zimmerman, 1881.

Aerobic bacteria, producing a violet chromoparous pigment, soluble in alcohol but not in chloroform. Motility and Gram reaction variable.

Type species, *Chr. violaceum* Bergonzini.

TRIBE 2. ERWINEAE

Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

GENUS 3. *ERWINIA*

Characters those of the tribe.

Type species *E. amylovora* (Burrill, 1883, p. 319; Trevisan, 1889, p. 19). Committee, 1917.

TRIBE 3. ZOPFEAE

Gram-positive rods, growing freely on artificial media. Not attacking carbohydrates.

GENUS 4. ZOPFIUS

Synonyms: *Bacterium* Ehrenberg, 1828 in part; *Bacillus* Cohn, 1872 in part; *Proteus* Hauser, 1885, in part.

Long rods occurring in evenly curved chains. Gram-positive. Motile. Proteus-like growth on media. Facultative anaerobes. Carbohydrates and gelatin not attacked, hydrogen sulfide not formed.

Type species, *Z. zopfii* (Kurth) Wenner and Rettger.

TRIBE 4. BACTEREAE

Gram-negative rods growing freely on artificial media. Generally forming acid from carbohydrates and often gas composed of CO₂ and H₂.

GENUS 5. PROTEUS HAUSER

Synonyms: *Spirulina* Hueppe, 1868; not *Spirulina* Turpin, 1827; *Liquidobacterium* Jensen, 1909.

Highly pleomorphic rods, filaments and curved cells being common as involution forms. Gram-negative. Actively motile. Characteristic amoeboid colonies on moist media. Liquefy gelatin rapidly and produce vigorous decomposition of proteins. Ferment glucose and sucrose (but usually not lactose), with formation of acid and gas (the latter being CO₂ only).

Type species, *P. vulgaris* Hauser.

GENUS 6. BACTERIUM

Synonyms: *Tyrothrix* Duclaux, 1879; *Actinobacter* Duclaux, 1882 in part; *Klebsiella* Trevisan, 1885 in part; *Kurthia*? Trevisan, 1885; *Gliscrobacterium* Malerba and Sanna Salaris, 1888; *Pneumobacillus*? Arloing, 1889; *Aerobacter* Beijerinck, 1900; *Salmonella* Lignieres, 1900; *Pyrobacillus* Koppányi, 1907.

Gram-negative, evenly staining rods. Often motile, with peritrichic flagella. Easily cultivable, forming grape-vine leaf or convex whitish surface colonies. Liquefy gelatin rarely. All forms except *B. alcaligenes* and the *B. abortus* group attack the hexoses and most species ferment a large series of carbohydrates. Acid formed by all gas (CO₂ and H₂) only by one series. Typically intestinal parasites of man and the higher animals although several species may occur on plants and one (*B. aerogenes*) is widely distributed in nature. Many species pathogenic.

TRIBE 5. LACTOBACILLEAE

Rods, often long and slender, Gram-positive, non-motile, without endospores. Usually produce acid from carbohydrates, as a rule lactic. When gas is formed, it is CO₂ without H₂. The organisms are usually somewhat thermophilic. As a rule microaerophilic; surface growth on media poor.

GENUS 7. LACTOBACILLUS

Synonyms: *Dispora*? Kern, 1882; *Tyrothrix*? Duclaux, 1882 in part; *Saccharobacillus*? van Laer, 1889; *Lactobacter* Beijerinck, 1901; *Streptobacillus* Rest and Khoury, 1902; *Brachybacterium* Troili-Peterson, 1903; *Cascobacterium* Jensen, 1909.

Generic characters those of the tribe.

The type species is *Lactobacillus caucasicus* (Kern?) Beijerinck.

TRIBE 6. PASTEURELLEAE

Gram-positive rods, showing bipolar staining. Parasitic forms of slight fermentative power.

GENUS 8. PASTEURELLA

Synonyms: *Octopsis?* Trevisan, 1885; *Coccobacillus* Gamaleia, 1888, not *Coccobacillus* Luebe, 1885; *Dicoccia?* Trevisan, 1889; *Diplobacillus?* Weichselbaum, 1887.

Aerobic and facultative. Powers of carbohydrate fermentation slight; no gas produced. Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in the lower animals.

The type species is *Pasteurella choleraegallinarum* (Flügge, 1886), Trevisan.

TRIBE 7. HEMOPHILEAE

Minute parasitic forms growing only in presence of hemoglobin, ascitic fluid or other body fluids.

GENUS 9. HEMOPHILUS

Synonyms: *Pyobacillus?* Koppanyi, 1907; *Diplobacillus* Morex, 1896, not *Diplobacillus* Weichselbaum, 1887.

Minute rod-shaped cells, sometimes thread-forming and pleomorphic, non-motile, without spores, strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood serum or ascitic fluid. Gram-negative.

The type species is *Hemophilus influenzae* (Pfeiffer, 1893, p. 357), Committee. 1917.

FAMILY VII: BACILLACEAE

Rods producing endospores, usually Gram-positive. Flagella when present peritrichic. Often decompose protein media actively through the agency of enzymes.

GENUS 1. BACILLUS

Synonyms: *Bactrella?* Morren, 1830; *Metallacter?* Perty, 1852; *Bacetridium* Davaine, 1868 in part; *Urobacillus* Miquel, 1879; *Pollendera* Trevisan, 1884; *Zopfella* Trevisan, 1885; *Streptobacter* Schroeter, 1886; *Cornilia* Trevisan, 1889 in part; *Bacterium* Ehrenberg, emended Migula, 1894 in part; *Bactridium* Fischer, 1895, not *Bactridium* Wallroth, 1832; *Bactrinium* Fischer, 1895; *Bactrillum* Fischer, 1895; *Endobacterium* Lehmann and Neumann, 1896; *Astasia* Meyer, 1898; *Fenobacter* Beijerinck, 1900; *Bacterius* Kendall, 1902 in part; *Aplanobacter* E. F. Smith, 1905 in part; *Semiclostridium* Massen, 1905; *Plennbakertum* Gonnermann, 1907; *Myxobacillus* Gonnermann, 1907; *Thermobacillus* Jensen, 1909; *Serratia* Vuillemin, 1913 in part, not *Serratia* Bizio, 1823.

Aerobic forms. Mostly saprophytes. Liquefy gelatin. Often occur in long threads and form rhizoid colonies. Form of rod usually not greatly changed at sporulation.

The type species is *Bacillus subtilis* Cohn.

GENUS 2. CLOSTRIDIUM

Synonyms: *Amylobacter* Trecul, 1865; *Cornilia* Trevisan, 1889 in part; *Granulobacter* Beijerinck, 1893; *Clostrillum* Fischer, 1895; *Clostrinium* Fischer, 1895; *Paracloster* Fischer, 1895; *Semi-clostridium* Massen, 1905; *Botulobacillus* Jensen, 1909;

Butyribacillus Jensen, 1909; *Cellulobacillus* Jensen, 1909; *Putribacillus* Jensen, 1909.

Anaerobes or micro-aerophiles. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

The type species is *Clostridium butyricum* Parzmowski.

IV. ARTIFICIAL KEY TO THE FAMILIES AND GENERA OF THE ACTINOMYCETALES AND EUBACTERIALES

- A. Typically filamentous forms. *Actinomycetaceae*
 - B. Mycelium and conidia formed. *Actinomyces*
 - BB. No true mycelium
 - C. Cells show branching
 - D. Gram-negative. *Actinobacillus*
 - DD. Gram-positive. *Erysipelothrix*
 - CC. Cells never branch. Gram-positive threads later fragmenting into rods
 - Leptotrichia*
- AA. Typically unicellular forms (although chains of cells may occur)
 - B. Spherical cells. *Coccaceae*
 - C. Parasitic forms. Cells in pairs, chains or irregular groups, never in packets. Generally active fermenters.
 - D. Cells in flattened coffee-bean-like pairs. Gram-negative. *Neisseria*
 - DD. Cells not as above. Gram-positive
 - E. Cells in lanceolate pairs or chains. Growth on media not abundant
 - F. Cells in lanceolate pairs. Inulin generally fermented. . . . *Diplococcus*
 - FF. Cells in chains. Inulin generally not fermented. *Streptococcus*
 - EE. Cells in irregular groups. Growth on media fairly vigorous
 - White or orange pigment. *Staphylococcus*
 - CC. Saprophytic forms. Chains occurring in zooglear masses in sugar solutions
 - Leuconostoc*
 - CCC. Saprophytic forms. Cells in irregular groups of packets not in chains. Fermentative powers low
 - D. Packets formed. *Sarcina*
 - DD. No packets
 - E. Yellow pigment. *Micrococcus*
 - EE. Red pigment. *Rhodococcus*
 - BB. Rods
 - C. Curved Rods. *Spirillaceae*
 - D. Short comma-like rods. One-three short flagella. *Vibrio*
 - DD. Long spirals, five-twenty flagella. *Spirillum*
 - CC. Straight rods
 - D. No endospores
 - E. Rods of irregular shape or showing branched or filamentous involution forms.
 - F. Animal parasites. Cells of irregular shape. Staining unevenly
 - Acid fast. *Mycobacterium*
 - GG. Not acid fast
 - H. Cells elongate, fusiform. *Fusiformis*
 - HH. Cells not fusiform, sometimes branching
 - I. Gram-positive. Slender, sometimes clubbed rods
 - Corynebacterium*

II. Gram-negative. Rods sometimes form threads.

Characteristic honey-like growth on potato.....*Pfeifferella*

FF. Not animal parasites. Cells staining unevenly and with branched or filamentous forms at certain stages. Never acid-fast

G. Metabolism simple, growth processes involving oxidation of alcohol or fixation of atmospheric nitrogen (latter in symbiosis with green plants)

H. Cells minute, symbiotes in roots of leguminous plants.*Rhizobium*HH. Oxidizing alcohol, branching forms common.....*Acetobacter*

GG. Not as above. Proteus-like colonies

H. Not attacking carbohydrates. Gram-positive.....*Zopfius*HH. Fermenting glucose and sucrose. Gram-positive.....*Proteus*

EE. Regularly formed rods.

F. Metabolism simple, growth processes involving oxidation of carbon, hydrogen or their simple compounds or the fixation of atmospheric nitrogen. *Nitrobacteriaceae*

G. Fixing nitrogen or oxidizing its compounds

H. Fixing nitrogen

Cells large; in soil.....*Azotobacter*

HH. Oxidizing nitrogen compounds

I. Oxidizing ammonia.....*Nitrosomonas*II. Oxidizing nitrites.....*Nitrobacter*

GG. Not as above

H. Oxidizing hydrogen.....*Hydrogenomonas*

HH. Not as above, using simpler carbon compounds

I. Oxidizing CO.....*Carboxydomonas*II. Oxidizing CH₄.....*Methanomonas*

FF. Not as above

G. Flagella usually present, polar. *Pseudomonadaceae*... *Pseudomonas*GG. Flagella when present peritrichic. *Bacteriaceae*H. Parasitic forms showing bipolar staining.....*Pasteurella*

HH. Not as above

K. Strict parasites growing only in presence of hemoglobin or ascitic fluid.....*Hemophilus*

II. Not as above

J. Water forms producing red or violet pigment

K. Pigment red.....*Erythrobacillus*KK. Pigment violet.....*Chromobacterium*

JJ. Not as above

K. Plant pathogens.....*Erwinia*

KK. Not as above

L. Gram-positive, forming large amount of acid from carbohydrates and sometimes CO₂ but no H₂... *Lactobacillus*LL. Gram-negative, forming H₂ as well as CO₂ if gas is produced.....*Bacterium*DD. Endospores present. *Bacillaceae*E. Aerobes.....*Bacillus*EE. Anaerobes.....*Clostridium*

The chart shown in Fig. 45, prepared by Macy of the University of Minnesota, shows the relation of the various divisions of this classification.

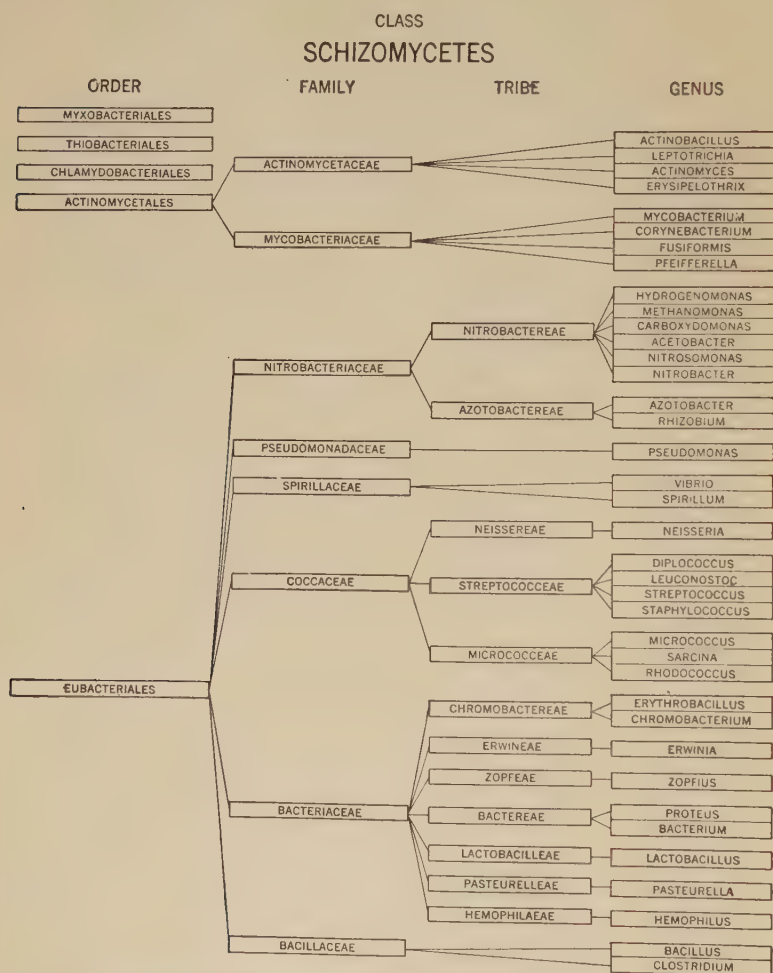


FIG. 45.—A Graphic Presentation of the Various Divisions of the Classification of Bacteria Prepared by a Committee of the Society of American Bacteriologists
(After Macy)

Bergey's Modification of the Classification Prepared by the Committee on the Characterization and Classification of Bacterial Types of the Society of American Bacteriologists.—The classification prepared by the Committee of the Society of American Bacteriologists was modi-

fied by Bergey in his Manual of Determinative Bacteriology. This classification was published in a book; Bergey and the committee which worked with him attempted to place most of the common species according to the revised classification.

CLASS SCHIZOMYCETES

Typically unicellular plants, cells usually small and relatively primitive in organization. The cells are of many shapes, spherical, cylindrical, spiral or filamentous; cells often united into groups, families or filaments; occasionally in the latter showing some differentiation among the cells, simulating the organization seen in certain of the blue-green filamentous algae. Multiplication typically by cell fission. Endospores are formed by some species of the Eubacteriales, conidia by some of the filamentous forms. Chlorophyll is produced by none of the bacteria (with the possible exception of a single genus). Many forms produce pigments of other types. The cells may be motile by means of flagella; some of the forms intergrading with the protozoa are flexuous, a few filamentous forms (as *Beggiatoa*) show oscillatory movement similar to that of certain blue-green algae (as *Oscillatoria*).

KEY TO THE ORDERS OF THE CLASS OF SCHIZOMYCETES

1. Simple and undifferentiated forms, without true branching. Occur as spheres, short or long straight rods, or as curved rods. Motile or non-motile. Endospore formation occurs in some species. Some species form pigment. Some species store reserve materials as volutin, glycogen or fat. Sulfur and iron are not stored as visible particles.

ORDER I. EUBACTERIALES

2. Specialized or differentiated forms, occurring as spheres, short or long spirals, or filamentous forms. Some of the longer forms occasionally show branching. Endospores are not formed. Some of the orders show segmentation of the terminal ends of filaments into short, specialized cells, conidia which are usually more resistant to external agencies than are the vegetative forms.

Many forms produce pigment, others contain bacteriopurpurin or bacteriochlorin or both. Some forms show definite granules of sulfur within the cytoplasm.

- a. Plant-like.
- b. Mold-like.

Cells rod-shaped or filamentous with decided tendency to true branching. Many pathogenic forms show distinct enlargement of the ends when growing in the animal body or even in culture media. Non-motile. Sulfur granules or bacteriopurpurin absent. Conidia may be formed.

FAMILY I. NITROBACTERIACEAE

Organisms usually rod-shaped. Cells motile or non-motile. Branched involution forms are produced. Endospores never formed. Obligate aerobes, capable of securing energy by the direct oxidation of carbon, hydrogen, sulfur, or nitrogen, or of simple, compounds of these. Non-parasitic (except in genus *Rhizobium*)—usually water or earth forms.

TRIBE 1. NITROBACTEREAE

Organisms deriving their life energy from oxidation of simple compounds of carbon and nitrogen (or of alcohol).

GENUS 1. HYDROGENOMONAS ORLA-JENSEN, 1909

Monotrichous short rods capable of growing in the absence of organic matter, and securing growth energy by the oxidation of hydrogen (forming water).

The type species is *Hydrogenomonas pantotropha* (Kaserer) Orla-Jensen.

GENUS 2. METHANOMONAS ORLA-JENSEN, 1909

Monotrichous short rods capable of growing in the absence of organic matter and securing growth energy by the oxidation of methane (forming carbon dioxide and water).

The type species is *Methanomonas methanica* (Söhngen) Orla-Jensen.

GENUS 3. CARBOXYDOMONAS ORLA-JENSEN, 1909

Autotrophic rod-shaped cells capable of securing growth energy by the oxidation of carbon monoxide (forming carbon dioxide).

The type species, *Carboxydomonas oligocarbophila* (Beijerinck and van Delden) Orla-Jensen.

GENUS 4. NITROSOMONAS WINOGRADSKY, 1892

Cells rod-shaped, motile, they possess polar flagella. Capable of securing growth energy by the oxidation of ammonia to nitrites. Growth on media containing organic matter scanty or absent.

The type species is *Nitrosomonas europaea* Winogradsky.

GENUS 5. NITROBACTER WINOGRADSKY, 1892

Cells rod-shaped, non-motile, not growing readily on organic media. Oxidizing nitrites to nitrates.

The type species is *Nitrobacter Winogradskyi*. Buchanan.

GENUS 6. NITROSOCOCCUS WINOGRADSKY, 1892

Large spherical organisms, showing no growth on ordinary culture media. Change nitrates to nitrites in soil and in suitable culture media.

The type species is *Nitrosococcus nitrosus* (Migula) Bergey et al.

GENUS 7. ACETOBACTER BEIJERINCK, 1901

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually on the surface of alcoholic solutions as obligate aerobes, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells may occur as involution forms.

The type species is *Acetobacter pasteurianum* (Hansen) Beijerinck.

GENUS 8. THIOBACILLUS BEIJERINCK, 1904

Small rod-shaped organisms deriving their energy from the oxidation of sulfides, thiosulfates or elementary sulfur, forming sulfur, persulfates and sulfates under acid or alkaline conditions and deriving their carbon from carbon dioxide or from bicar-

bonates and carbonates in solution; some are obligate and some facultative autotrophic. One species is anaerobic.

The type species is *Thiobacillus thioparus* Beijerinck.

TRIBE II. AZOTOBACTEREAE

Nitro-fixing bacteria

GENUS 9. AZOTOBACTER

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates. Motile or non-motile; when motile, with tuft of polar flagella. Obligate aerobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen.

The type species is *Azotobacter chroococcum* Beijerinck.

GENUS 10. RHIZOBIUM FRANK, 1889

Minute rods, motile when young. Branching forms abundant and characteristic when grown under suitable conditions. Obligate aerobes, capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates and in the absence of compounds of nitrogen. Produce nodules upon the roots of leguminous plants.

The type species is *Rhizobium leguminosarum* Frank.

FAMILY II. COCCACEAE ZOPF, 1884

Cells in their free conditions, spherical; during division somewhat elliptical. Division in one, two or three planes. If the cells remain in contact after division they are frequently flattened in the plane of division, and occur singly, in pairs, tetrads, chains, packets or in irregular masses. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates. Pigment often produced. Motility rare. Endospores absent.

TRIBE 1. STREPTOCOCCEAE TREVISAN, 1889

Parasites (thriving only or best on or in the animal body) except genus *Leuconostoc*. Grow well under anaerobic conditions. Many forms grow with difficulty on serum-free media, none very abundantly. Planes of fission usually parallel, producing pairs or short or long chains, never packets. Generally stain by Gram. Produce acid but no gas in glucose and generally in lactose broth. Pigment, if any, white or orange.

GENUS 1. DIPLOCOCCUS WEICHSELBAUM, 1886

Parasites, growing poorly, or not at all, on artificial media. Cells usually in pairs of somewhat elongated cells, encapsulated, sometimes in chains. Gram-positive. Fermentative powers high, most strains forming acid in glucose, lactose, sucrose and inulin.

Type species, *Diplococcus pneumoniae* Weichselbaum.

GENUS 2. STREPTOCOCCUS ROSENBAACH, 1884

Chiefly parasites. Cells in pairs or in short or long chains, never in packets. Generally Gram-positive. Capsules rarely formed. Do not form zoogloeal masses. Grow as effused, translucent, often small isolated colonies on agar streak. In stab cultures little surface growth is developed. Many carbohydrates are fermented

with formation of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates. Some species lake blood, others produce methemoglobin, while a smaller number are without action on blood.

The type species is *Streptococcus pyogenes* Rosenbach.

GENUS 3. LEUCONOSTOC VAN TIEGHEM, 1878

Saprophytes, usually growing in cane-sugar solutions. Cells in chains or in pairs united in zooglear masses. Some types, at least, are Gram-negative.

The type species is *Leuconostoc mesenteroides* (Cienkowski) van Tieghem.

TRIBE II. NEISSEREA COMMITTEE S. A. B., 1920

Strict parasites, some species failing to grow or growing poorly on usual culture media. Gram stain varies. Growth fairly abundant on serum media. Cells normally in pairs.

GENUS 4. NEISSERIA TREVISAN, 1885

Characters, those of tribe.

Type species, *N. Gnorrrhaee* Trevisan.

GENUS 5. GAFFKYA TREVISAN, 1885

Parasitic organisms, occurring in the animal body and in special media as tetrads, while in ordinary culture media they occur in pairs and irregular masses. Gram-positive.

Type species, *Gaffkya tetragena* (Gaffky) Trevisan.

TRIBE III. MICROCOCCAE DE TONI AND TREVISAN, 1889

Facultative parasites or saprophytes. Thrive best under aerobic conditions. Grow well on artificial media, producing abundant surface growth. Planes of fission often at right angles occurring singly and in pairs and in cell aggregates of groups or packets. Generally stained by Gram. Many species form yellow or red pigment.

GENUS 6. STAPHYLOCOCCUS ROSENBAACH, 1884

Usually parasitic, cells occur singly and in pairs and in irregular groups, rarely in packets. Usually Gram-positive. Growth fair to good on the surface of artificial media. As a rule carbohydrates are fermented with the formation of acid. Gelatin commonly liquefied. Nitrates may or may not be reduced. (Produce hemolysis on blood agar.) Pigment white or orange, or less commonly lemon-yellow.

The type species is *Staphylococcus aureus* Rosenbach.

GENUS 7. MICROCOCCUS COHN, 1872

Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or packets). Generally stained by Gram. Growth on agar usually abundant, some species form no pigment but others form yellow or less commonly, orange pigment. Dextrose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied, but not rapidly.

The type species is *Micrococcus luteus* (Schröter) Cohn.

GENUS 8. SARCINA GOODSIR, 1842

Saprophytes and facultative parasites. Division occurs, under favorable conditions, in three planes, producing regular packets. Usually Gram-positive. Growth

on agar abundant, usually with formation of yellow or orange pigment. Dextrose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied. Nitrates may or may not be reduced.

The type species is *Sarcina ventriculi* Goodsir.

GENUS 9. RHODOCOCCLUS ZOPF, 1891

Saprophytes. Cells in groups or in irregular packets. Usually Gram-positive. Abundant growth with red pigment on surface of culture media. Slight acid from dextrose; none from lactose. Gelatin liquefied by some species. Nitrates usually reduced to nitrites but not to ammonia.

The type species is *Rhodococcus rhodochrous* Zopf.

FAMILY III. SPIRILLACEAE MIGULA, 1894

Cells elongate, more or less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous. Usually without endospores. As a rule motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

GENUS 1. VIBRIO MÜLLER, 1786

Cells short bent rods, rigid, single or united into spirals. Motile by means of a single (rarely two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aerobic facultative anaerobic. No endospores. Usually Gram-negative. Water forms; a few parasites. Type species, *Vibrio comma* (Schröter).

GENUS 2. SPIRILLUM EHRENBURG, 1830

Cells rigid, rods of varying thickness, length, and pitch of spiral, forming either long screws or portions of a turn. Usually motile by means of a tuft of polar flagella (to 20) which are mostly half-circular, rarely curled. The flagella occur at one or both poles; the number varies greatly and is difficult to determine. Found in water and putrid infusions.

The type species is *Spirillum undula* (Müller) Ehrenberg.

FAMILY IV. BACTERIACEAE COHN, 1872

Rod-shaped cells without endospores. Usually Gram-negative. Motile or non-motile. Metabolism complex, amino-acids being utilized, and generally carbohydrates.

TRIBE 1. CHROMOBACTEREAE COMMITTEE S. A. B., 1920

Water and soil bacteria producing a red, yellow, violet or blue-green pigment.

GENUS 1. SERRATIA, BIZIO, 1823

Small aerobic rods, producing a red or pink pigment, usually a lipochrome. Gram-negative. Motile or non-motile.

Type species, *Serratia marcescens* (Ehrenberg) Committee.

GENUS 2. FLAVOBACTERIUM BERGEY ET AL., 1923

Rods of medium size, occurring in water and soil forming a yellow to orange pigment on culture media characterized by feeble powers of attacking carbohydrates,

occasionally forming acid from hexoses but no gas. Motile or non-motile. Generally Gram-negative.

The type species is *Flavobacterium aquatilis* (Frankland) Bergey et al.

GENUS 3. CHROMOBACTERIUM BERGONZONI, 1881

Aerobic bacteria, producing a violet chromoparous pigment, soluble in alcohol, but not in chloroform.

Type species, *Chromobacterium violaceum* Bergonzoni. Bergey et al.

GENUS 4. PSEUDOMONAS MIGULA, 1894

Principally water and soil bacteria producing a water-soluble pigment which diffuses through the medium as green, blue or yellowish green. Motile or non-motile. Gram-negative.

The type species is *Pseudomonas aeruginosa* (Schröter) Migula.

TRIBE II. CELLULOMONADEAE BERGEY ET AL., 1923

Short rods occurring in soil, having the property of digesting cellulose. Motile or non-motile. Chromogenic or non-chromogenic. Growth on ordinary culture media often not vigorous. Gram-negative.

GENUS 5. CELLULOMONAS BERGEY ET AL., 1923

Small rods, with rounded ends, non-spore-forming, motile or non-motile, occurring in oil and having the property of digesting cellulose.

Type species, *Cellulomonas biazotea* (Kellerman) Bergey et al.

TRIBE III. ACHROMOBACTEREAE BERGEY ET AL., 1923

Rods, small to medium size, occurring principally in water and soil. Form no pigment on agar or gelatin but may produce a brownish growth on potato. Cultural characters variable. Some species form acid in simple sugars, others are without action on carbohydrates. Motile or non-motile. Gram-negative.

GENUS 6. ACHROMOBACTER BERGEY ET AL., 1923

Non-pigment forming (at most no pigment formed on agar or gelatin) rods, occurring in water and soil. Motile or non-motile. Gram-negative.

Type species, *Achromobacter liquefaciens* (Eisenberg) Bergey et al.

TRIBE IV. LACTOBACILLEAE COMMITTEE S. A. B., 1920

Rods, often long and slender. Gram-positive. Non-motile. Without endospores. Generally produce acid, as a rule lactic, from carbohydrates. When gas is formed it is CO₂ without H₂. The organisms are usually somewhat thermophilic. As a rule microaerophilic. Surface growth on media is poor.

GENUS 7. LACTOBACILLUS BEIJERINCK, 1901

Generic character are those of the tribe.

The type species is *Lactobacillus caucasicus* (Kern) Beijerinck.

TRIBE V. ERWINEAE COMMITTEE S. A. B., 1920

Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid or acid and gas usually formed in carbohydrate media. Motile, or non-motile, Gram-negative.

GENUS 8. ERWINIA COMMITTEE S. A. B., 1917

Motile rods, possessing peritrichous flagella. The rods are white, and growth usually is white, although a few species form pigment.

Type species, *Erwinia amylovora* (Burrill) Committee S. A. B., 1920.

GENUS 9. PHYTONOMAS ET AL., 1923

Rods, yellow or white, motile or non-motile, the motile species possessing either mono- or lophotrichous flagella. May or may not form yellow pigment. The type species is *Phytomonas campestris* (Pammel) Bergey et al.

TRIBE VI. KURTHIEAE TREVISAN, 1889

Gram-positive rods, growing freely on artificial media. Do not attack carbohydrates.

GENUS 10. KURTHIA TREVISAN, 1885

Long rods occurring in evenly curved chains. Gram-positive. Motile. Proteus-like growth on media. Facultative anaerobic. Carbohydrates and gelatin not attacked. Hydrogen sulfid not formed.

The type species is *Kurthia zopfii* (Kurth) Trevisan.

TRIBE 7. BACTEREAE COMMITTEE, S. A. B., 1920

Gram-negative rods occurring commonly in the intestinal tract of animals. Grow well on artificial media. Many species attack carbohydrates forming acid or acid and gas. Relatively few species liquefy gelatin. When motile, the flagella are peritrichous.

GENUS 11. ESCHERICHIA CASTELLANI AND CHALMERS, 1919

Motile or non-motile rods, commonly occurring in the intestinal canal of normal animals. Attack numerous carbohydrates forming acid and frequently acid and gas. Do not produce acetyl-methyl-carbinol.

Type species *Escherichia coli* (Escherich) Castellani and Chalmers.

GENUS 12. AEROBACTER BEIJERINCK, 1900

Motile or non-motile rods, commonly occurring in the intestinal canal or normal animals. Produce acetyl-methyl-carbinol. The type species is *Aerobacter aerogenes* (Escherich) Beijerinck.

GENUS 13. PROTEUS HAUSER, 1885

Highly pleomorphic rods. Filamentous and curved rods are common as involution forms. Gram-negative. Generally actively motile, possessing peritrichous flagella. Produce characteristic ameoboid colonies on moist media and decompose proteins. Ferment dextrose and sucrose, but not lactose. Do not produce acetyl-methyl-carbinol.

The type species is *Proteus vulgaris* Hauser.

GENUS 14. *SALMONELLA* LIGNIÈRES, 1900

Motile forms. Generally occur in the intestinal canal of animals in various types of acute, inflammatory conditions. Attack numerous carbohydrates with the formation of both gas and acid. In general do not form acetyl-methyl carbinol.

Type species, *Salmonella suispestifer* (Kruse) Lignières.

GENUS 15. *EBERTHELLA* BUCHANAN, 1918

Motile or non-motile rods, generally occurring in the intestinal canal of man, usually in different forms of enteric inflammation. Attack a number of carbohydrates with the formation of acid but no gas. Do not form acetyl-methyl-carbinol.

Type species, *Eberthella typhi* (Eberth-Gaffky) Buchanan.

GENUS 16. *ALCALIGINES* CASTELLANI AND CHALMERS, 1919

Motile or non-motile rods, generally occurring in the intestinal canal of normal animals. Do not form acetyl-methyl-carbinol. Do not ferment any of the carbohydrates.

Type species, *Alcaligines fecalis* Castellani and Chalmers.

TRIBE VIII. PASTEURELLEAE CASTELLANI AND CHALMERS, 1919

Gram-negative rods showing bipolar staining. Parasitic forms with slight fermentative powers. There is a single genus.

GENUS 17. *PASTEURELLA* TREVISAN, 1887

Aerobic, facultative. Powers of carbohydrate fermentation slight; no gas produced. Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in the lower animals.

The type species is *Pasteurella avicidea* (Kitt) Trevisan.

TRIBE IX. KLEBSIELLEAE TREVISAN, 1887

Short rods, somewhat plump with rounded ends, mostly occurring singly. Encapsulated. Non-motile. Gram-negative. Ferment a number of carbohydrates with the formation of acid and gas. Encountered principally in the respiratory tract of man. Aerobic, growing well on ordinary culture media.

GENUS 18. *KLEBSIELLA* TREVISAN, 1885

The generic characters are those of the tribe.

The type species is *Klebsiella pneumoniae* Trevisan.

TRIBE X. HEMOPHILEAE COMMITTEE S. A. B., 1920

Minute parasitic forms growing only in the presence of hemoglobin, ascitic fluid or other body fluids, or in the presence of certain growth accessory substances found in sterile unheated plant tissue (potato). Non-motile. Gram-negative.

GENUS 19. *HEMOPHILUS* COMMITTEE S. A. B., 1917

Minute rod-shaped cells, sometimes thread forming and pleomorphic. Non-motile. Strict parasites growing best (or only) in the presence of hemoglobin, and in general requiring blood serum, ascitic fluid or certain growth accessory substances.

Type species, *Hemophilus influenzae* (Pfeiffer) Committee S. A. B.

GENUS 20. *DIALISTER* BERGEY ET AL., 1923

Minute rod-shaped cells, occurring singly, in pairs and in short chains. Non-motile. Strictly parasites. Growth occurs only under anaerobic conditions in media containing fresh sterile tissue or ascitic fluid.

The only species known is *Dialister pneumosintes* (Olitsky and Gates) Bergey et al.

TRIBE XI. BACTEROIDEAE CASTELLANI AND CHALMERS, 1919

Motile or non-motile rods, without endospores. Show good growth on ordinary culture media; without pigment formation. Obligate anaerobes.

GENUS 21. *BACTEROIDES* CASTELLANI AND CHALMERS, 1919

The characters of the genus are those of the tribe.

The type species is *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers.

FAMILY V. BACILLACEAE FISCHER, 1895

Rods producing endospores, usually Gram-positive. Flagella when present peritrichous. Often decompose protein media actively through the agency of enzymes.

GENUS 1. *BACILLUS* COHN, 1872

Aerobic forms. Mostly saprophytes. Generally liquefy gelatin. Often occur in long threads and form rhizoid colonies. Form of rod usually not greatly changed at sporulation. Young organisms are Gram-positive.

The type species is *Bacillus subtilis* (Ehrenberg) Cohn.

GENUS 2. *CLOSTRIDIUM* PRAZMOWSKI, 1880

Anaerobes or micro-aerophiles. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

The type species is *Clostridium butyricum* Prazmowski.

ORDER II. ACTINOMYCETALES BUCHANAN, 1918

Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings and clubbed or irregular shapes. No pseudoplasmodium. No deposits of free sulfur or iron. No bacteriopurpurin. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Some species are parasitic in animals or plants. Not water forms. Complex proteins frequently required. As a rule strongly aerobic (except for some species of *Actinomyces* and the genera *Fusiformia* and *Leptotrichia*) and oxidative. Growth on culture media often slow; some genera show mold-like colonies.

FAMILY I. ACTINOMYCETACEAE BUCHANAN, 1918

Filamentous forms often branched and sometimes forming mycelia. Conidia sometimes present. Some species are parasitic.

GENUS 1. ACTINOBACILLUS BRUMPT, 1910

Filament formation, resembling streptobacilli. In lesions no mycelium formed, but at peripheries finger-shaped branched cells are visible. Gram-negative. Not acid-fast.

Type species, *Actinobacillus lignieresii* Brumpt.

GENUS 2. LEPTOTRICHIA TREVISAN, 1879

Thick, long, straight or curved filaments, unbranched, frequently clubbed at one end and tapering to the other. Gram-positive when young. Filaments fragment into short, thick rods. Anaerobic or facultative. No aerial hyphae or conidia. Parasites or facultative parasites.

Type species, *Leptotrichia buccalis* (Robin) Trevisan.

GENUS 3. ACTINOMYCES HARZ, 1877

Organism growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Sometimes parasitic, with clubbed ends of radiating threads conspicuous in lesions in animal body. Some species are microaerophilic or anaerobic. Non-motile.

The type species is *Actinomyces bovis* Harz.

GENUS 4. ERYSIPELOTHRIX ROSENBAACH, 1909

Rod-shaped organisms with a tendency to the formation of long filaments which may show branching. The filaments may also thicken and show characteristic granules. No spores. Non-motile. Gram-positive. Do not produce acid. Microaerophilic. Usually parasitic.

The type species is *Erysipelothrix rhusiopathiae* (Migula) Holland.

FAMILY II. MYCOBACTERIACEAE CHESTER, 1901

Parasitic forms. Rod-shaped, frequently irregular in form, but rarely filamentous and with only slight and occasional branching. Often stain unevenly (showing variations in staining reaction within the cell). No conidia formed.

GENUS 1. MYCOBACTERIUM LEHMANN AND NEUMANN, 1896

Slender rods which are stained with difficulty, but when once stained are acid-fast. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even branched forms. Growth on media slow. Aerobic. Several species pathogenic to animals.

The type species is *Mycobacterium tuberculosis* (Koch) Lehmann and Neumann.

GENUS 2. *CORYNEBACTERIUM* LEHMANN AND NEUMANN, 1896

Slender, often slightly curved, rods with a tendency to club and pointed forms. with branching forms in old cultures. Barred, uneven staining. Not acid-fast, Gram-positive. Non-motile. Aerobic. No endospores. Some pathogenic species produce a powerful exotoxin. Characteristic snapping motion is exhibited when cells divide.

The type species is *Corynebacterium diphtheriae* (Klebs-Löffler) Lehmann and Neumann.

GENUS 3. *FUSIFORMIS* HOELLING, 1910

Obligate parasites. Anaerobic or microaerophilic. Cells frequently elongate fusiform, staining somewhat unevenly. Filaments sometimes formed; non-branching. Non-motile. No spores formed. Growth in laboratory media freeble.

The type species is *Fusiformis termitidis* Hoelling.

GENUS 4. *PFEIFFERELLA* BUCHANAN, 1918

Non-motile rods, slender, Gram-negative, staining poorly, sometimes forming threads and showing a tendency toward branching. Gelatin may be slowly liquefied. Do not ferment carbohydrates. Growth on potato characteristically honey-like.

The type species is *Pfeifferella mallei* (Löffler) Buchanan.

ORDER III. *CHLAMYDOBACTERIALES* BUCHANAN, 1918

Filamentous bacteria, alga-like, typically water forms, frequently sheathed, without true branching although false branching may be present. The sheath is frequently impregnated with iron. Conidia may be developed, but never endospores. Sulfur granules or bacteriopurpurin never present. Mature cells or filaments not motile nor protozoan-like.

The order contains a single family.

FAMILY I. *CHLAMYDOBACTERIACEAE* MIGULA, 1894ORDER IV. *THIOBACTERIALES* BUCHANAN, 1918

Cells various, typically containing either granules of free sulfur, or bacteriopurpurin, or both, usually growing best in the presence of hydrogen sulfid. The cells are plant-like, not protozoan-like, not producing a pseudoplasmodium or a highly developed resting stage. Spores are rarely or never formed.

ORDER V. *MYXOBACTERIALES* BUCHANAN, 1918

Motile, rod-like organisms multiplying by fission, secreting a gelatinous base and forming a pseudoplasmodium-like aggregation before passing into a more or less highly developed cyst-producing, resting stage in which the rods may become encysted in groups without modification, or may be converted into spore masses.

There is one family only, *Myxobacteriaceae*.

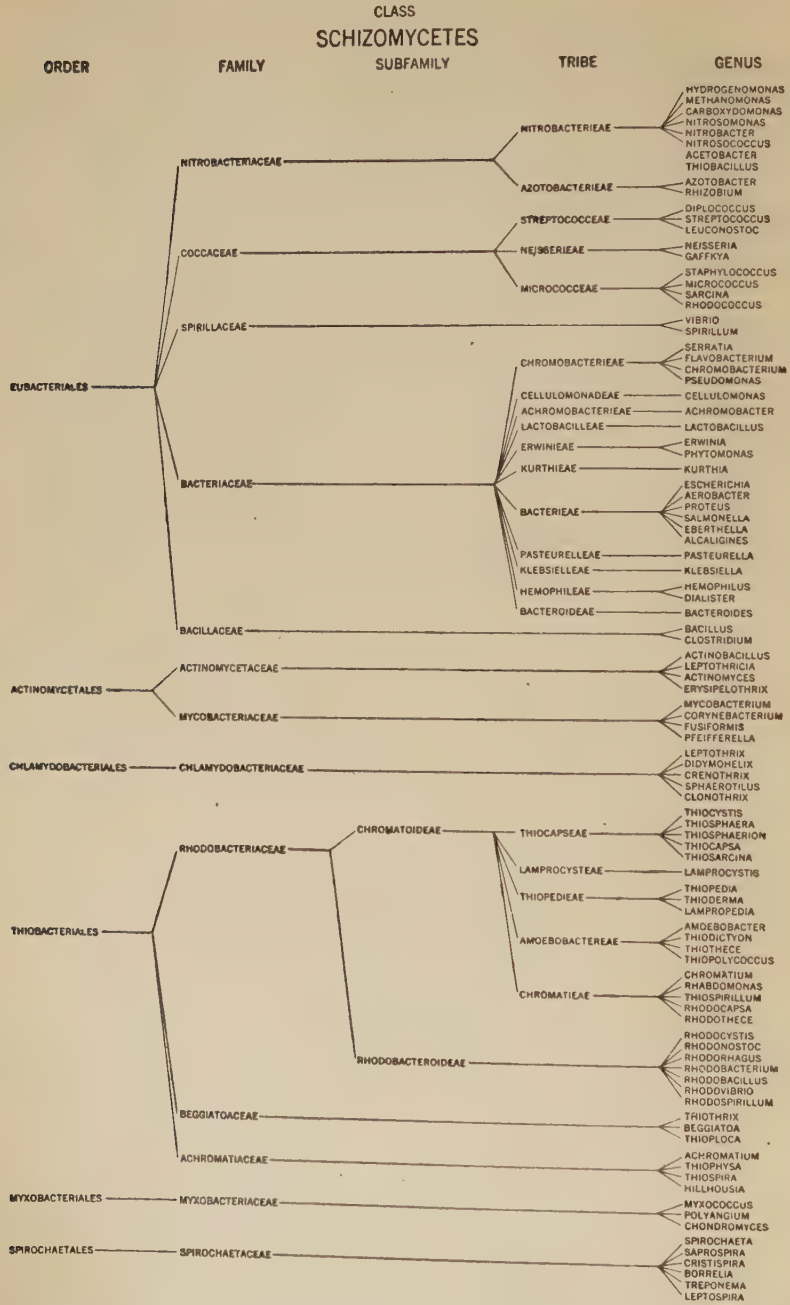


FIG. 46.—A Graphic Presentation Showing the Various Divisions of Bergey's Modification of the Committee of the S. A. B. Classification (Prepared by Wallace).

NAMES OF SOME OF THE MORE COMMON
BACTERIA ACCORDING TO OLDER CLASS-
IFICATIONS.

abortus, *Bacillus*
abortus-equi, *Bacillus*
acidi lactici, *Bacterium*
acidophile aerogenes, *Bacillus*
acidophilus, *Bacillus*
acne, *Bacillus*
adhaerens, *Bacillus*
aerogenes, *Bacillus*
aerogenes capsulatus, *Bacillus*
aertrycke, *Bacillus*
aeruginosus, *Bacillus*
agilis, *Micrococcus*
agile, *Bacterium*
agri, *Bacillus*
albolactis, *Bacillus*
albus, *Micrococcus*
alcalescens, *Bacterium*
alcaligines, *Bacillus*
amethystinus, *Bacillus*
amylobacter, *Bacillus*
amyloruber, *Bacillus*
amylovorus, *Bacillus*
anatum, *Bacterium*
anthracis, *Bacillus*
anthracis-symptomatici, *Bacillus*
anthracoides, *Bacillus*
aquatilis, *Bacillus*
arborescens, *Bacillus*
aromafaciens, *Bacterium*
asterosporus, *Bacillus*
ascendens, *Bacterium*
astheniae, *Bacterium*
aurantiaca, *Sarcina*
aurantiacus, *Micrococcus*
aureus, *Staphylococcus*
avisepticus, *Bacillus*
beijerincki, *Bacillus*
bibulus, *Bacillus*
bifermentans, *Bacillus*
bifidus, *Bacillus*

NAMES OF SOME OF THE MORE COMMON
BACTERIA ACCORDING TO BERGEY'S
MODIFICATION OF THE CLASSIFICA-
TION PROPOSED BY THE COMMITTEE
OF THE SOCIETY OF AMERICAN BAC-
TERIOLOGISTS.³

Alcaligines abortus
Salmonella abortus equi

Lactobacillus acidophile aerogenes
Lactobacillus acidophilus
Fusiformis acne
Bacillus adhaerens
Aerobacter aerogenes
Clostridium Welchii
Salmonella aertrycke
Pseudomonas aeruginosa
Rhodococcus agilis
Achromobacter agile
Bacillus agri
Bacillus albolactis
Micrococcus albus
Escherichia alcalescens
Alcaligines fecalis
Chromobacterium amethystinum
Clostridium butyricum
Serratia amyloiber
Erwinia amylovora
Salmonella anatum
Bacillus anthracis

Bacillus megaterium
Flavobacterium aquatilis
Flavobacterium arborescens
Achromobacter aromafaciens
Bacillus asterosporus
Acetobacter ascendens
Escherichia astheniae
Sarcina aurantiaca
Micrococcus aurantiacus
Staphylococcus aureus
Pasteurella avicida
Azotobacter beijerincki
Cellulomonas bibula
Clostridium fermentans
Bacteroides bifidus

³ A longer and more complete list of microorganisms with the old and new names was prepared by Dr. Bergey and published in Gould's Medical Dictionary, 2d Edition, P. Blakiston's Son & Co., Philadelphia, 1928.

bipolaris, *Bacillus*
 botulinus, *Bacillus*
 bovisepiticus, *Bacillus*
 brassicae, *Bacterium*
 brevis, *Bacillus*
 bronchisepticus, *Bacillus*
 bucchalis, *Bacillus*
 bulgaricus, *Bacillus*
 Bütschlii, *Bacillus*
 butyricus, *Bacillus* (Hueppe)
 butyricus, *Bacillus* (Botkin)
 campestris, *Pseudomonas*
 candicans, *Bacillus*
 candicans, *Micrococcus*
 capsulatus, *Bacillus* (Sternberg)
 capsulatus-mucosus, *Bacillus*
 carotovorus, *Bacillus*
 casei, *Bacterium*
 casei, *Micrococcus*
 catarrhalis, *Micrococcus*
 caucasicus, *Bacillus*
 centrosporus, *Bacillus*
 cereus, *Bacillus*
 chauvei, *Bacillus*
 chinense, *Bacterium*
 cholerae, *Bacterium*
 cholerae-asiaticae, *Spirillum*
 cholerae-gallinarum, *Bacillus*
 cholerae-suis, *Bacillus*
 cinnebareus, *Micrococcus*
 circulans, *Bacillus*
 citreus, *Staphylococcus*
 citreus, *Micrococcus*
 citri, *Bacillus*
 cloacae, *Bacillus*
 coagulans, *Bacillus*
 cohaerans, *Bacillus*
 coli, *Bacillus* (Escherich)
 communior, *Bacterium*
 conglomeratus, *Micrococcus*
 cyanogenes, *Bacillus*
 Danysz, *Bacillus*
 Delbrücki, *Bacillus*
 denitrificans, *Bacillus*
 diphtheriae, *Bacillus*
 dysenteriae, *Bacillus* (Shiga)
 edematis, *Bacillus*
 enteritidis, *Bacillus*
 enteritidis, sporogenes, *Bacillus*
 fecalis-alcaligines, *Bacillus*

Bacillus cohaerens
Clostridium botulinum
Pasteurella bovisepitica
Bacillus megaterium
Flavobacterium brevis
Alcaligines bronchisepticus
Micrococcus buccalis
Lactobacillus bulgaricus

Bacillus lacticolus
Bacillus sphaericus Neide
Phytomonas campestris
Achromobacter candicans
Micrococcus candicans
Klebsiella capsulatus

Erwinia carotovora
Lactobacillus casei
Micrococcus casei
Neisseria catarrhalis
Lactobacillus caucasicus
Bacillus centrosporus
Bacillus cereus
Clostridium chauvei
Aerobacter chinense
Vibrio comma
Vibrio comma
Pasteurella avicida
Salmonella suispestifer
Rhodococcus cinnebareus
Bacillus circulans
Staphylococcus citreus
Staphylococcus citreus
Photomonas citri
Aerobacter cloacae
Bacillus coagulans
Bacillus cohaerans
Escherichia coli
Escherichia communior

Bacillus syncyaneus

Lactobacillus delbrückii
Achromobacter agile
Corynebacterium diphtheriae
Eberthella dysenteriae

Salmonella enteritidis
Clostridium Welchii
Alcaligines fecalis

<i>finum</i> , <i>Bacterium</i>	<i>Cellulomonas fima</i>
<i>flavus</i> , <i>Micrococcus</i>	<i>Micrococcus flavus</i>
<i>fluorescens</i> , <i>Bacillus</i>	<i>Pseudomonas fluorescens</i>
<i>fluorescens</i> , <i>Proteus</i>	<i>Pseudomonas jaegeri</i>
<i>freudenreichii</i> , <i>Micrococcus</i>	<i>Micrococcus freudenreichii</i>
<i>fusiformis</i> , <i>Bacillus</i>	<i>Bacillus fusiformis</i>
<i>gallinarum</i> , <i>Bacillus</i> (Klein)	<i>Salmonella gallinarum</i>
<i>gasoformans</i> , <i>Bacillus</i>	<i>Achromobacter gasoformans</i>
<i>globigii</i> , <i>Bacillus</i>	<i>Bacillus globigii</i>
<i>gonorrhoeae</i> , <i>Diplococcus</i>	<i>Neisseria gonorrhoeae</i>
<i>graveolens</i> , <i>Bacillus</i>	<i>Bacillus graveolens</i>
<i>grünthali</i> , <i>Bacterium</i>	<i>Escherichia grünthali</i>
<i>hayducki</i> , <i>Bacillus</i>	<i>Lactobacillus hayducki</i>
<i>Hoffmanni</i> , <i>Bacillus</i>	<i>Corynebacterium Hoffmanni</i>
<i>hyacinthi</i> , <i>Bacillus</i>	<i>Phytomonas hyacinthi</i>
<i>icteroides</i> , <i>Bacillus</i>	<i>Salmonella icteroides</i>
<i>indicus</i> , <i>Bacillus</i>	<i>Serratia indica</i>
<i>influenzae</i> , <i>Bacillus</i>	<i>Hemophilus influenzae</i>
<i>intracellularis-meningitidis</i> , <i>Diplococcus</i>	<i>Neisseria intracellularis</i>
<i>involutus</i> , <i>Diplococcus</i>	
<i>kützingianum</i> , <i>Bacillus</i>	<i>Acetobacter kützingianum</i>
<i>lactici-acidi</i> , <i>Bacillus</i>	
<i>lacticus</i> , <i>Streptococcus</i>	<i>Streptococcus lactis</i>
<i>lactis-acidi</i> , <i>Bacillus</i>	<i>Lactobacillus lactisacidi</i>
<i>lactis aerogens</i> , <i>Bacillus</i>	<i>Aerobacter aerogenes</i>
<i>lactis</i> , <i>Bacillus</i> (Flügge)	<i>Bacillus lactis</i>
<i>lactis-viscosus</i> , <i>Bacillus</i>	<i>Achromobacter viscosum</i>
<i>laterosporus</i> , <i>Bacillus</i>	<i>Bacillus laterosporus</i>
<i>leguminosarum</i> , <i>Rhizobium</i>	<i>Rhizobium leguminosarum</i>
<i>leprae</i> , <i>Bacillus</i>	<i>Mycobacterium leprae</i>
<i>levans</i> , <i>Bacillus</i>	<i>Aerobacter levans</i>
<i>lindneri</i> , <i>Bacterium</i>	<i>Acetobacter lindneri</i>
<i>lividus</i> , <i>Bacillus</i>	<i>Chromobacterium lividum</i>
<i>lutea</i> , <i>Sarcina</i>	<i>Sarcina lutea</i>
<i>luteus</i> , <i>Micrococcus</i>	<i>Micrococcus luteus</i>
<i>Mallei</i> , <i>Bacillus</i>	<i>Pfeifferella mallei</i>
<i>megaterium</i> , <i>Bacillus</i>	<i>Bacillus megaterium</i>
<i>melonis</i> , <i>Bacillus</i>	<i>Erwinia melonis</i>
<i>melitensis</i> , <i>Bacillus</i>	<i>Alcaligines melitensis</i>
<i>mesentericus</i> , <i>Bacillus</i>	<i>Bacillus mesentericus</i>
<i>methanicus</i> , <i>Bacillus</i>	<i>Methanomonas methanica</i>
<i>morgani</i> , <i>Bacterium</i>	<i>Salmonella morgani</i>
<i>murisepticus</i> , <i>Bacillus</i>	<i>Erysipelothrix murisepticae</i>
<i>mycoides-resens</i> , <i>Bacillus</i>	<i>Serratia rosea</i>
<i>neopolitanus</i> , <i>Bacillus</i>	<i>Escherichia neapolitana</i>
<i>niger</i> , <i>Bacillus</i>	<i>Bacillus niger</i>
<i>nitrificans</i> , <i>Bacillus</i>	<i>Achromobacter nitrificans</i>
<i>of Achalme</i> <i>Bacillus</i>	<i>Clostridium Welchii</i>
<i>of Boas-Oppler</i> , <i>Bacillus</i>	<i>Lactobacillus boas-oppleri</i>
<i>of Ducrey</i> , <i>Bacillus</i>	<i>Hemophilus ducreyii</i>

- of Klebs-Loeffler, *Bacillus*
 of Koch-Weeks, *Bacillus*
 of Morax-Axenfeld, *Bacillus*
 of Schottmüller, *Bacillus*
 of Shiga, *Bacillus*
 of Sternberg, *Bacillus*
oleae, *Bacillus*
oleraceae, *Bacillus*
oligocarbophilus, *Bacillus*
oxytocum, *Bacterium*
ozaeanae, *Bacillus*
panis, *Bacillus*
pantotrophus, *Bacillus*
paradysenteriae, *Bacillus*
paratyphi, *Bacillus* (Schottmüller)
paratyphosus B, *Bacillus*
paratyphosus A, *Bacillus*
pasteurianum, *Clostridium*
perfringens, *Bacillus*
pestis, *Bacillus*
pertussis, *Bacillus*
pestis-caviae, *Bacillus*
phaseoli, *Bacillus*
phosphorescens, *Bacillus*
plymouthensis, *Bacillus*
pneumoniae, *Bacillus*
prodigiosus, *Bacillus*
proteus-mirabilis, *Bacillus*
proteus-fluorescens, *Bacillus*
proteus vulgaris, *Bacillus*
pruni, *Bacillus*
pseudo-anthraxis, *Bacillus*
pseudo-coloides, *Bacterium*
pseudo-diphtheriae, *Bacillus*
pseudo-tetanicus, *Bacillus*
psittacosis, *Bacillus*
pullorum, *Bacillus*
putrificus, *Bacillus*
pyocyaneus, *Pseudomonas*
pyogenes, *Streptococci*
pyogenes-foetidus, *Bacillus*
radicicola, *Bacillus*
ramosus, *Bacillus*
rosaceus, *Micrococcus*
roseus, *Micrococcus*
rubefaciens, *Bacillus*
ruber, *Bacillus*
rubrum, *Spirillum*
runinatus, *Bacillus*
saccharobutyricus, *Bacillus*
Cornybacterium diphtheriae
Hemophilus conjunctividis
Hemophilus lacunatus
Salmonella schottmülleri
Eberthella dysenteriae
Erwinia oleracea
Carboxydomonas oligocarbophila
Aerobacter oxytocum
Klebsiella ozaenae
Lactobacillus panis
Hydrogenomonas pantotrophus
Eberthella paradysenteriae
Salmonella paratyphi
Salmonella schottmülleri
Salmonella paratyphi
Clostridium butyricum
Clostridium welchii
Pasteurella pestis
Hemophilus pertussis
Phytomonas phaseoli
Serratia plymouthensis
Klebsiella pneumoniae
Serratia marcescens
Proteus mirabilis
Pseudomonas jaegeri
Proteus vulgaris
Phytomonas pruni
Bacillus megaterium
Escherichia pseudocoloides
Cornybacterium pseudodiphthericum
Bacillus pseudotetanicus
Salmonella psittacosis
Salmonella pullorum
Clostridium putrificum
Pseudomonas aeruginosa
Streptococci pyogenes
Eberthella pyogenes
Rhizobium radicicola
Bacillus mycoides
Rhodococcus rosaceus
Rhodococcus roseus
Serratia rubefaciens
Serratia ruber
Spirillum rubrum
Bacillus ruminatus
Clostridium butyricum

sanguinarum, *Bacillus*
schafferi, *Bacterium*
shigae, *Bacillus*
simplex, *Bacillus*
smegmatis, *Bacillus*
solonacearum, *Bacillus*
sporogenes, *Bacillus*
subtilis, *Bacillus*
supestifer, *Bacillus*
suisepcticus, *Bacillus*
terminalis, *Bacillus*
tetani, *Bacillus*
tetragenus, *Micrococcus*
tuberculosis, *Bacillus*
tumefaciens, *Bacillus*
tumescens, *Bacillus*
typhi, *Bacillus*
typhi-abdominalis, *Bacillus*
typhi-murium, *Bacillus*
typhosus, *Bacillus*
ureae, *Micrococcus*
varians, *Micrococcus*
vekanda, *Bacterium*
ventriculi, *Sarcina*
vesiculosum, *Bacterium*
violaceus, *Bacillus*
vulgaris, *Bacterium*
vulgatus, *Bacillus*
Welchii, *Bacillus*
Xerosis, *Bacillus*
xylinum, *Bacillus*
zenkeri, *Bacillus*
zopfii, *Bacillus*

Eberthella sanguinaria
Escherichia schafferi
Eberthella dysenteriae
Bacillus simplex
Mycobacterium smegmatis
Phytomonas solonaceara
Clostridium sporogenes
Bacillus subtilis
Salmonella supestifer
Pasteurella suisepctica
Bacillus terminalis
Clostridium tetani
Gaffkyia tetragenus
Mycobacterium tuberculosis
Phytomonas tumefaciens
Bacillus tumescens
Eberthella typhi
Eberthella typhi
Salmonella typhimurium
Eberthella typhi
Micrococcus ureae
Micrococcus varians
Escherichia vekanda
Sarcina ventriculi
Escherichia vesiculosum
Chromobacterium violaceum
Proteus vulgaris
Bacillus vulgatus
Clostridium welchii
Corynebacterium xerosis
Acetobacter xylinum
Kurthia zenkeri
Kurthia zopfii

GROUPS OF BACTERIA

Bacteriologists for the sake of convenience have grouped bacteria which have some property in common. It would be unnecessary in a book of this sort to attempt a lengthy presentation of such groups or the members of these groups. Consequently, only a few of the larger and more important ones will be discussed with a few species in each case. These will be discussed at varying lengths since certain groups, such as the colon-typhoid groups, are treated fully in easily accessible reference texts. The aerobic spore-forming group is not so treated and therefore is given more space here. Only the more significant characteristics of the various species will be given.

Aerobic Spore-forming Bacteria.—This group includes those bacteria which form endospores and, grow aerobically on laboratory media. They are very common organisms in nature and are usually encountered in any study of bacterial populations and species of foods, soil, etc. There seems to some confusion in identification and separation of these forms. Below are the names of some of the better known species. This group has received intensive study at Johns Hopkins University by Ford and his colleagues from whose publications the following notes on this group have been prepared.

Bacillus cereus.—This organism was first described by the Franklands in 1887. Since that time the organism has been described under several different names. It is widely distributed in nature. Lawrence and Ford characterized the species as follows:

Morphology. Regular bacilli with homogeneous protoplasm and rounded ends, in young cultures measuring about 0.75 by 2.25 to 4 microns. Many of the organisms show peculiar refractile bodies of various sizes as the cultures get older, presenting a characteristic appearance. The nature of these bodies is not clear as they do not give reactions for starch or volutin. They can usually be differentiated from the beginning spores. On glucose agar the bacilli are thicker and longer, measuring 0.75 to 1 by 3 to 6 microns. Here the entire protoplasm of the organism is converted into the bodies mentioned above. They are globular, highly refractile, and are often as thick as the organism.

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early on both plain and glucose agar, often appearing within twenty-four hours or even in less time. They may be central in position, excentric or even sub-terminal but the latter location of the spore is rare. The spores are usually wider than the organisms from which they spring and thus bulge the rods slightly. The free spores retain for some time their protoplasm at the ends, usually in equal amounts. Often, however, the protoplasm is greater at one end than at the other and the spore then has a characteristic appearance like an enlarged mesentericus spore. The free spores are cylindrical, soon shed their protoplasm and measure 0.5 to 0.75 by 1.125 to 1.5 microns.

Agar slant. Abundant, thick, white mealy growth along the line of inoculation, sometimes with arborescent edges. In older cultures the growth is much thicker, yellowish-white and may show pellucid areas surrounded by more highly refractive patches.

Agar stab. Little growth along line of inoculation, but luxuriant surface growth spreading over entire surface of agar and extending to the walls of the tube.

Agar colonies. Round, raised, dense, highly refractive surface colonies. If slight amount of water of condensation is present the colonies may be amoeboid. Under low power the colonies consist of dense central nuclei with spreading peripheries made up of numerous curling and parallel chains. The colonies are soft and easily lifted from the agar. Deep colonies punctiform, stellate or rhizoid. Under low power they are fuzzy, irregular and may resemble a chestnut burr.

Litmus glucose agar slant. Thick, yellowish-white growth along the line of inoculation and spreading out over entire surface. The medium is acidified and the

growth is sometimes distinctly yellow. Typical cultures rapidly decolorize the litmus and then become alkaline and the agar turns deep blue. Occasionally the cultures are less active alkali-producers and the medium remains permanently acid. Such cultures, however, can usually be stimulated to alkali production by plating and they then give characteristic growths.

Glucose agar colonies. Surface colonies round or bizarre, heaped up, with irregular margins, smaller than plain agar colonies. Under low power granular, with dense central nuclei and irregular margins, showing fine parallel strands. Deep colonies small irregular or round. Under low power they consist of dense central nuclei with fine, irregular or parallel strands in the periphery.

Gelatin stab. Uniform growth along entire line of inoculation with a liquefaction also along entire line. The liquefaction becomes cup-shaped or sacculated with a surface scum. It is rapid, and frequently in two days the entire gelatin tube is liquefied.

Gelatin colonies. Loosely filamentous colonies with dense, central nuclei and spreading irregular margins, often very thin, edges entire. Gelatin liquefied rapidly.

Broth. Very turbid in twenty-four hours with no scum except occasionally a slight ring growth. In two days a heavy friable scum is produced which is entirely precipitated within a short time. The medium gradually clears while a heavy flocculent precipitate is deposited.

Peptone. Very turbid in twenty-four hours. Scum appears usually on the second day and is soon precipitated. It is like that produced in broth, but is more friable.

Potato. Thick, white, mealy growth in a few days becoming yellowish or brown with a discoloration of the potato. This brownish growth may become very moist and slimy and is occasionally measley, but never vermiform. It never assumes an appearance similar to that seen with cultures of *Bacillus subtilis* or *Bacillus vulgaris*.

Litmus milk. With the majority of cultures peptonization begins immediately and progresses rapidly in three zones. Surface zone is amber, middle zone violet, the lowest zone blue. Peptonization continues until the entire milk tube is converted into an amber fluid with a slight sediment. Milk does not coagulate. With some cultures the three-zone appearance does not show, but the milk is gradually changed to a muddy gray-colored fluid. Eventually, however, the same clear amber-colored fluid is produced.

Blood serum. Thick, white, dry, smooth growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl and arm. Friable scum forms which is soon precipitated. Turbidity gradually disappears and a flocculent precipitate is deposited. Reaction highly acid.

Saccharose. Abundant growth in bowl and arm. Filmly and friable scum forms which is soon precipitated. In many cultures the reaction is acid. Reaction alkaline in the majority of cultures.

Lactose. Turbidity in bowl with scum formation. Arm clear. Reaction alkaline.

Thermal death point. The spores survive steaming one hour in the Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus subtilis: This is one of the oldest of described species. It is widely distributed in nature, being easily isolated from water, soil, milk, etc. Lawrence and Ford described the species as follows:

Morphology. Small, thin, homogeneous bacilli measuring 0.375 by 1.5 to 2.5

microns in twenty-four-hour agar cultures. Somewhat thicker and longer on glucose agar, measuring 0.5 by 1.5 to 4 microns. Does not usually form threads on this medium.

Motility. Sluggishly motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early appearing within twenty-four hours on plain and glucose agar. They arise in the center or towards one end of the rods and are slightly greater in diameter than the rods, thus causing a distinct bulging. The free spores may retain bits of protoplasm at each end, often unequal in amount, giving the spore a characteristic appearance. Such spores measure about 0.5 by 0.875 micron. The spores rapidly lose their protoplasm, become more oval and measure about 0.5 by 0.75 micron.

Agar slant. Weakly refractive, glassy, membranous growth along line of inoculation, later spreading out over entire surface of agar. The surface is usually dry and hard though in old cultures it becomes soft and smeary, but it is always firmly attached to the agar from which it cannot be scraped off.

Agar stab. Little growth along the line of inoculation but a spreading, dry, membranous growth on the surface of the agar, extending to the wall of the tube.

Agar colonies. Surface colonies weakly refractive, spreading concentrically or in amoeboid fashion from small dense nuclei. Under low power the edges may be complete or finely crenate. If water of condensation is present one or two colonies frequently overgrow the entire plate. Under the low power the colonies are homogeneous and granular or irregular and gyrose. The deep colonies are punctiform and under the lower power lichenlike with irregular margins myceleoid in character. The colonies are usually membranous dry, hard, and glassy, and can be separated from the agar only with great difficulty.

Glucose litmus agar slant. Highly refractive growth verrucose or vesicular, with milky liquid in vesicles, not spreading. Parts of growth show distinct red pigment. Acid is produced in twenty-four hours, but is replaced by alkali in about ten days, medium turning deep blue.

Litmus glucose agar colonies. Irregular, spreading, bizarre surface colonies, usually more luxuriant than plain agar colonies. Under low power, irregular with entire edges or fuzzy, with myceleoid outgrowths from dense central nuclei. Deep colonies slightly irregular or punctiform. Under low power irregular myceleoid with filamentous edges. Medium first acidified, then made alkaline.

Gelatin stab. Slow growth along line of inoculation and rather slow, cup-shaped, surface liquefaction with scum production

Gelatin colonies. Surface colonies round, homogeneous, spreading, thin and granular. Deep colonies yellowish brown, highly refractive. Under low power granular. Colonies may also show dense central nuclei and thin myceloid filamentous growth extending in every direction through the medium. Gelatin liquefied.

Broth. Single isolated pellicles appear on the surface in twenty-four hours. In forty-eight hours these unite to form a thin branching scum, which gradually becomes more dense and tough. Medium grows turbid in first twenty-four hours, but later clears. Scum is precipitated as a whole in about ten days. This manner of scum formation is characteristic of *Bacillus subtilis*.

Peptone. Turbidity in the first twenty-four hours and gradual clearing with a flocculent precipitate. Scum on the surface formed in the same manner as on broth, but not so dense or tough. The pellicles often show chains and branching figures. Frequently the scum has a delicate pink color after about five days' growth.

Potato. Growth on potato characteristic. It is luxuriant and warty, having the appearance of many large and small dewdrops scattered along the line of inoculation. In forty-eight hours a pink pigment collects on top of this growth and persists. In older cultures a decided rose-red line in the substance of the potato marks the limit of the growth. In ten days the vesicles dry down and only a reddish-brown dry growth remains on the discolored medium. Later the growth is moist and sticky.

Litmus milk. No change in twenty-four hours and sometimes none in forty-eight hours except that the milk becomes more alkaline. In three days the medium begins to clear from the surface, the deeper parts remaining unchanged. Clearing progresses slowly, the supernatant fluid persisting as a grayish, pinkish or yellowish muddy medium. After a month at room temperature the medium may become very alkaline and turn deep blue-purple. Milk never coagulates.

Blood serum. Vesicular, dewdrop growth with pink color often very marked, in twenty-four hours. Vesicles dry down, eventually leaving a hard wrinkled growth. Medium is not liquefied.

Fermentation tubes. Glucose. Turbidity in bowl and arm. Scum formation like that seen in broth. Highly acid.

Saccharose. Turbidity in bowl and arm with a fragile scum forming from pellicles in about two days. Acid production but not so marked as in glucose.

Lactose. Turbidity in bowl and extending up in the arm to the level of the medium in the bowl. Rest of the arm clear. Dense tough scum. Reaction alkaline.

Thermal death point. Spores survive steaming $1\frac{1}{4}$ hours in the Arnold sterilizer. Survive autoclaving up to and including 19 pounds pressure but usually destroyed by 20 pounds pressure.

Bacillus vulgaris: This organism is commonly known as the "potato bacillus." Some authors have thought it to be identical with *Bacillus subtilis*. However, Lawrence and Ford believed that, if proper tests were used, these species could be separated. They characterized the organism as follows:

Morphology. Small homogeneous organisms usually distinctly larger than *Bacillus subtilis*, measuring 0.5 by 2 to 3 microns. Occasionally short forms 1.125 and long forms measuring 4 microns are seen on plain agar. On glucose agar the organisms are thicker and much longer, measuring often nearly 0.75 micron in thickness and 5 microns in length.

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early, appearing in twenty-four hours on plain and glucose agar. They arise in the center or toward one end of the rods but do not ordinarily bulge the rods appreciably. When free they are elongated and flattened and retain tags of protoplasm at each end. At times the protoplasm at one end is greater in amount than at the other. Such spores measure about 0.5 by 1.125 microns. As they lose their protoplasm they become cylindrical, measuring about 0.5 by 1 micron. In general the spores are about the same width as the vegetative rods or only very slightly wider.

Agar slant. Moist, profuse, thick growth on agar, easily lifted or brushed from the surface of the medium with the platinum wire. Growth is usually white or cream-white, spreads but little from the line of inoculation and is whitest at the edge where it is heaped up. When water of condensation washes over the agar many small, round colonies develop apart from the main growth. In some strains the agar growth is

dry and fine wrinkles develop but the growth can always be lifted from the agar with a platinum loop.

Agar stab. Little growth along line of inoculation but rather dry wrinkled rooty growth spreads over the surface of the agar.

Agar colonies. Surface colonies round, waxy, highly refractive or spreading and amoeboid, with greatest refraction at the edge of the advancing growth, where colonies are thickest. Under low power of the microscope edges entire. Deep colonies punctiform, round or elliptical. Under low power they are irregular, brown, slightly granular with entire or fuzzy edges.

Litmus glucose agar slant. Characteristic appearance. Luxuriant dry, brown and abundantly wrinkled growth develops within twenty-four to forty-eight hours. The medium is acidified. After a few days the growth usually becomes moist and the wrinkles are obliterated while the medium becomes alkaline and turns deep blue.

Litmus glucose agar colonies. Superficial colonies are thick, highly refractive, waxy, with entire edges or spreading with irregular edges. They soon become dry and wrinkled in the center. Under low power opaque with entire edges. Deep colonies are punctiform, round, oval or irregular with crenated margins. Under low power opaque with irregular margins. Medium acidified at first, then turned alkaline.

Gelatin. Stab gives cup-shaped and surface liquefaction with heavy scum production.

Gelatin colonies. Colonies round with highly refractive centers occasionally showing beautiful concentric rings. Under the low power the colonies have a granular appearance. Medium liquefied.

Broth. Turbidity within twenty-four hours and scum formation usually on second day. Medium gradually clears.

Peptone. Turbidity within twenty-four hours. Thin fragile scum after the lapse of several days. Medium gradually clears.

Potato. Characteristic appearance. Thick, white, gray or pink folds or wrinkles are formed within twenty-four to forty-eight hours often covering entire cut surface of potato. Later these folds dry down to a brown, reticulate mass. Potato usually discolored.

Litmus milk. Slight clearing of the milk just beneath the cream layer usually appears in twenty-four hours. This reaction rapidly intensifies with the production of a clear fluid colored deep Chinese blue or purple. No acid production or coagulation. As the milk gets older, complete peptonization occurs with the formation of a clear amber-colored fluid.

Blood serum. Thin dry abundant growth usually smooth, but sometimes wrinkles and pink. Growth later becomes moist and gives a suggestion of liquefaction.

Fermentation tubes. Glucose. Luxuriant growth in bulb gradually extending into the closed arm. An abundant scum is formed which may be quite wrinkled. Reaction acid.

Saccharose. Growth luxuriant in bowl but scanty in arm. Very thin scum may be formed after several days, but this may be lacking. Reaction varies from slight to marked acidity.

Lactose. Abundant growth in bowl with late scum production. No growth in closed arm. Reaction alkaline.

Thermal death point. Organisms survived heating in broth in the Arnold sterilizer for one hour. Survived autoclaving up to and including 19 pounds pressure, but were destroyed by 20 pounds pressure.

Bacillus mesentericus: This species was described by Flügge in 1886. It has the following characteristics (Lawrence and Ford).

Morphology. Organisms about the same in morphology as *Bacillus mesentericus-vulgatus*. On agar cultures in twenty-four hours they are homogeneous rods measuring 0.5 by 1.5 to 3 microns. Sometimes shorter forms predominated in the cultures, a little over a micron in length. On glucose agar they are thicker and longer, measuring 0.75 by 2 to 6 microns, with many long forms measuring 6 to 8 microns in length.

Motility. Active motility, progressive and rotatory, in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form in twenty-four hours on plain and on glucose agar. By the end of forty-eight hours they are very abundant. They appear in the center or towards one end of the rods and do not bulge the organism appreciably. The free spores are cylindrical and may retain equal bits of protoplasm at each end or this protoplasm may be unequal in amount, giving a characteristic appearance to the spore. They measure about 0.5 by 1.125 microns. They rapidly lose their protoplasm and become slightly more oval, measuring 0.5 by 0.75 micron.

Agar slant. Soft white or cream-white growth somewhat translucent when old, spreading but little from the line of inoculation except in the presence of water of condensation. Easily lifted from the agar. Edges of growth irregular or serrate. Growth does not become dry or wrinkled.

Agar stab. Little growth along line of puncture, luxuriant growth on surface.

Agar colonies. Superficial colonies round highly refractive with entire edges, or spreading and amoeboid. Under low power opaque with crenated edges. Deep colonies round and regular. Under low power slightly granular with crenated margins.

Litmus glucose agar slant. Thick, abundant, white or cream-white to yellow growth spreading along the line of inoculation. Medium first turns acid, but as growth becomes older it again becomes deep blue.

Litmus glucose agar colonies. Superficial colonies round, highly refractive, with entire edges or spreading and amoeboid with densest part of the growth along the advancing edge. Under low power of the microscope edges crenated. Deep colonies round or oval and under low power slightly granular with crenated margins. Medium first acidified and then made alkaline.

Gelatin stab. Cup-shaped or surface liquefaction and scum production.

Gelatin colonies. Colonies dense with liquefaction centers and granular ring at the edges of a cup-shaped liquefaction.

Broth. Turbidity and a rather fragile scum appears late. Medium then clears.

Peptone. Turbidity with small patches of surface growth. Medium soon clears.

Potato. Growth abundant, moist, brown with finely wrinkled or lichen-like appearance in the majority of instances. At times the fine wrinkling is lacking and only a thick, moist, brown, mealy growth is produced.

Litmus milk. Slow peptonization with the production of a lilac color turning to amber. In a few weeks digestion is complete and only a white sediment is left behind. No acidification. No coagulation.

Blood serum. Thin, white, dry, at times finely wrinkled growth which later becomes yellowish and moist. Suggestion of liquefaction, but this is never complete.

Fermentation tubes. Glucose. Turbidity and scum in bulb and turbidity in closed arm. Reaction acid.

Saccharose. Turbidity in open bulb and usually no scum. Turbidity in closed arm. Reaction acid.

Lactose. Turbidity in open bulb. No scum. Arm clear. Reaction alkaline.

Thermal death point. Spores survive one hour's heating in Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus mycoides: This species is also widely distributed in nature and, according to Lawrence and Ford, has the following characteristics:

Morphology. In young cultures six to eight hours old on plain agar the organisms are homogeneous with square ends and measure usually a little more than 0.5 micron in width by 3 to 6 microns in length. They are distinctly thinner and longer than *Bacillus cereus*. As the organisms mature the protoplasm appears more granular and a characteristic arrangement in short and long chains is seen. They then resemble the anthrax bacillus. On glucose agar the bacilli are thicker, 0.75 to 1 micron, and usually about the same length. On this medium the protoplasm is converted into globular bodies which do not take the stain and which are similar to those seen in *Bacillus cereus*. In certain instances the organisms seem to be made up of a network of fine strands in which the globular bodies hang suspended. Often the chains are curled or curved upon themselves. Old cultures show an abundance of swollen involution forms, which seem to have a skein-like arrangement.

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early, appearing first as small refractile bodies in the centers or toward one end of the organisms usually at the end of twenty-four to forty-eight hours. Gradually the organisms swell and the spores at the same time increase in size and at this stage a long chain of organisms each containing a spore may often be seen. The protoplasm soon disintegrates, leaving a rim about the spore which is round or oval or slightly rectangular. Such spores are more definitely elongated and may measure 0.75 to 1 by 2 microns. The spores often remain attached to each other in short or long chains. The spores vary more in size than do others of this group and may show small forms 0.375 to 0.5 to 1 and large forms measuring 1.125 by 2 lying side by side.

Agar slant. Filamentous rhizoid growth spreading from the line of inoculation and extending into the agar. This growth is at first glassy and glistening, but later grows dull and soft. Appearance on agar characteristic.

Agar stab. Faint arborescent growth along line of inoculation with a surface development in concentric zones.

Agar colonies. Surface colonies spread from dark dense nuclei and show dense, rhizoid peripheries extending into the agar on all sides. Under low power the periphery of the colony is found to be composed of parallel strands of growth. Deep colonies have almost the same appearance and always exhibit the spreading peripheral myceloid outgrowths.

Litmus glucose agar. Thin membranous myceloid growth later becoming branched and reticulate. Growth at first moist and white, later becoming pale yellow. Medium first acidified and then turned deep blue.

Litmus glucose agar colonies. Surface colonies thin, round or irregular. Under low power found to consist of masses of matted filaments with usually dense central nuclei, from which single or parallel strands extend into the agar in every direction for long distances. Deep colonies exhibit the same small, punctiform and matted myceloid growth, under low power. Medium first acidified and then made alkaline.

Gelatin stab. Filamentous growth along line of inoculation with surface liquefaction.

Gelatin colonies. Colonies consist of dense central nuclei with matted edges from which long strands emerge. The colonies present a peculiar appearance like a chest-nut burr.

Broth. No turbidity but a firm scum forms which is soon precipitated.

Peptone. No turbidity, but a flocculent suspension and a firm scum which is soon precipitated.

Potato. Mealy white, later becoming brownish.

Litmus milk. Slow peptonization to an amber-colored fluid. No acidification. No coagulation.

Blood serum. Dry, myceloid interlacing luxuriant growth. No liquefaction.

Fermentation tubes. Glucose. Flocculent growth in bowl and arm. Scum forms and is soon precipitated. Reaction acid.

Saccharose. Flocculent in bowl and in arm. Scum is formed and precipitated. Some cultures produce moderate acidity. Others produce no acid.

Lactose. Growth in open bulb with a slight extension into arm. Scum formed and soon precipitated. Reaction alkaline.

Thermal death point. Spores survived one hour in the Arnold sterilizer and 15 pounds pressure in the autoclave. Destroyed by 16 pounds pressure.

Bacillus megatherium: This is another species which is widely distributed in nature. Lawrence and Ford characterized it as follows:

Morphology. These are the largest of the spore-bearing organisms. On plain agar in young cultures, from eight to twenty-four hours old, they are long and thick with homogeneous or slightly granular protoplasm, measuring 0.75 to 1.25 by 3 to 9 microns. On glucose agar they are even thicker measuring 1.25 to 1.5 microns in width. On both media long forms occur, but especially on glucose agar. These may measure 30 to 45 microns in length and may show homogeneous protoplasm without evident segmentation. The protoplasm of the organism is at first homogeneous, but by the end of twenty-four to forty-eight hours it is converted into a mass of globular bodies resistant to the stains. These globular bodies are clear, highly refractile, bulge the organism somewhat, and are quite numerous, six to eight appearing in each rod. They thus give the organism a peculiar and characteristic appearance. They show most markedly on glucose agar but are also present on plain agar where they can best be demonstrated by decolorizing an overstained preparation. Their nature is not clear as they do not take any special bacterial stains. Shadow or transparent forms appear in *Bacillus megatherium* early, both on plain and glucose agar. These measure 1.1235 to 1.5 by 4 to 10 microns, take the stain very faintly and show peculiar bodies of agglomerated protoplasm at the sides or sometimes at the ends. These transparent forms are often thicker and longer and may even measure 2 by 40 to 45 microns. Occasionally they are distinctly oval with rounded ends measuring about 1.5 by 4 microns and show a small bunch of cytoplasm at the side. When these forms are in chains they are exactly like the original pictures of De Bary.

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed abundantly on plain agar in twenty-four hours and on glucose agar in forty-eight hours. They appear in the center or slightly towards one end of the rods and are usually of the same diameter but may be slightly thicker. Sometimes two spores seen to arise in one rod but these may possibly be in a

rod just prior to division. In general each rod has a single spore. The spores occasionally lie obliquely in the rods. Frequently two spores are at opposite ends of rods lying in juxtaposition and these may remain attached in chains and present a characteristic appearance. The free spores retain protoplasm at the ends for some time. When this is unequal in amount the spore has somewhat the shape of a tennis racket and handle. The free spores are oval to cylindrical and measure 0.75 to 1.125 by 1.5 to 2 microns. They are often flattened on one side, having an appearance described as kidney-shaped or reniform. The spores show great variations in size, more so than those of the other members of this group.

Slant agar. Thick, raised, soft, white or cream-colored growth which shows a pink tinge by reflected light, with many small, minute pellucid areas. As the cultures get older the growth becomes pale yellow.

Agar stab. Slight growth along line of inoculation, heaped up and spreading slightly on surface. Later surface growth becomes slightly pinkish.

Agar colonies. Surface colonies round, thick, white or cream-colored, highly refractive, turning pale yellow or yellowish-brown in old cultures. Under low power slightly granular, brownish yellow, with entire margins. Deep colonies punctiform. Under low power round or irregular with entire edges, brown and granular.

Litmus glucose agar slant. Thick, luxuriant growth along line of inoculation, at first white and then pale yellow or cream-colored. Medium is first acidified, but later becomes alkaline and changes from a dark blue to a smoky brown while the growth becomes a dark gray or smoky brown.

Litmus glucose agar colonies. Large, round, raised surface colonies, cream-colored to pale yellow, with heaped-up central nuclei. Under low power dark, slightly granular with entire edges. Deep colonies punctiform. Under low power dark irregular, bizarre, with entire edges. Medium is acidified and then made alkaline.

Gelatin stab. Funnel-shaped liquefaction. No scum.

Gelatin colonies. Round colonies with concentric zones of growth. Under low power cloudy central nuclei with filamentous peripheries.

Broth. Turbidity but no scum.

Peptone. Turbidity but no scum.

Potato. Thick, white, mealy growth later becoming pale or cream yellow.

Litmus milk. No change within twenty-four hours, then a gradual peptonization with the production of a port-wine-colored fluid. No acidification. No coagulation.

Blood serum. Thick, white, moist, heavy growth cream-white to yellow in color. No liquefaction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Saccharose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Lactose. Slight turbidity in open bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores withstood one hour steaming in Arnold sterilizer and 18 pounds pressure in the autoclave. Killed by 19 pounds pressure.

Besides the forms described fully above the following are also members of the aerobic spore-forming group; descriptions may be found in the papers by Ford and his colleagues:

Bacillus fusiformis

Bacillus ruminatus

Bacillus cohaerens

Bacillus panis

Bacillus terminalis
Bacillus graveoleus
Bacillus tumescens

Bacillus adhaerens
Bacillus centrosporus
Bacillus brevis

Most of the aerobic spore-forming bacteria are non-pathogenic. One species, *Bacillus anthracis*, is very pathogenic for animals.

Bacillus anthracis: This organism was one of the first species to be studied in pure culture. It was studied by Koch and Pasteur when they were beginning to work in bacteriology. It is pathogenic to man and certain animals. The characteristics of anthrax are given in Chapter XXVI. The species has the following characteristics:

Morphology. Slender rods 1 to 1.2 by 2 to 8 or 20 microns in length; rods sometimes in chains.

Spores. Formed under favorable conditions.

Staining reactions. Gram-positive; stains easily with ordinary dyes.

Gelatin. Small opaque colonies edge fringed with wavy filaments; gelatin readily liquefied.

Agar colonies. Similar to those on gelatin, a hairy wavy colony.

Broth. Growth occurs in flocs which settle to the bottom of the tube leaving a clear supernatant fluid.

Pathogenesis. Pathogenic for many animals.

Anaerobic Spore-forming Bacteria.—For convenience, the bacteria-forming endospores and growing anaerobically have been grouped together. Both of these characteristics are salient ones and form a good basis for grouping. The group may be subdivided on the basis of pathogenicity which will be used here for a division into two parts. This division is merely for convenience. Into this group fall some of nature's most active microorganisms.

Non-Pathogenic Species.—The non-pathogenic anaerobic spore-bearing bacteria have not received as much study as the pathogenic species. However, some of them are very important in industrial fermentations and in certain significant reactions in nature. Several of the more important species will be described since the more uncommon ones may be investigated in other books.

Clostridium putrificum (*Bacillus putrificus*): This is a widespread putrefactive anaerobe discovered in 1890 by Bienstock. It has received much study recently at the hands of a number of different investigators. Reddish published a description of the organism. We may use some of his data for characterizing the species.

Morphology. Long, slender rods, often curved; long filaments frequently in old cultures; $0.5-0.7 \times 8.0\mu$.

Spores. Round and strictly terminal; very large in proportion to the rod. Spores not seen until after the tenth day in egg-meat mixture. Spores remain in the rods for several days.

Staining reactions. Weakly Gram-positive in young cultures. Stains well with ordinary aniline dyes.

Glucose agar colonies. Small, delicate and almost transparent; edge irregularly but not deeply indented; granular structure.

Milk. Slow precipitation of casein followed by digestion.

Broth. Scant growth.

Gelatin. Rapid liquefaction.

Fermentation reactions. Glucose only slightly attacked; other carbohydrates not fermented

Pathogenicity. Non-pathogenic for mice and guinea pigs.

Clostridium sporogenes (*Bacillus sporogenes*): This anaerobic spore-forming bacterium is widespread in nature and has been said to be a contaminant of many strains of anaerobic bacteria. There are apparently several very closely related strains of the organism. The type species has the following characteristics.

Morphology. Rods, usually single but sometimes occurring in chains of a few units. Motile by means of peritrichic flagella.

Sporulation. Spore central or polar. Easily formed in all media.

Agar colony. Hairy colony, opaque.

Milk. Coagulation followed by peptonization.

Meat. Putrid odor, blackening with gas.

Gelatin. Liquefied.

Oxygen relations. Anaerobic.

Fermentation reactions. Acid and gas formed in most of the common sugars.

Pathogenicity. Non-pathogenic.

Pathogenic Species.—This subgroup contains some of the most active organisms with which man has to cope. They form very powerful toxins which show a special affinity for living protoplasm.

Clostridium botulinum (*Bacillus botulinus*): This organism forms one of the most potent toxins known. The poisoning, botulism, which it causes, is discussed in a later chapter. *Clostridium botulinum* is a very active organism, being able to decompose proteins and carbohydrates vigorously. Many of the statements in books published before the increased study of botulism in America around 1920 are inaccurate. The characteristics are much better known since then than before.

Morphology. Large pleomorphic, sluggishly motile rods, occur singly and in chains.

Sporulation. Oval, subterminal spores formed; very resistant to destructive agents, especially heat; spores large, giving clostridial shaped cells.

Agar. Colonies may be regular but usually irregular. Round and flat; semi-opaque; blood agar colonies are round, minute, irregular.

Gelatin. Liquefied.

Milk. Scant growth; slowly coagulated and digested with gas production.

Meat. Scant growth.

Fermentation reactions. Glucose, glycerol and salicin fermented; lactose, sucrose, inulin and sugar-free broth not fermented.

Pathogenicity. Pathogenic in large doses; the toxin, however, is active in minute quantities.

Three types of *Clostridium botulinum* are known; they are known as Types A, B, and C. While these types are not easily differentiated by cultural reactions they are by means of seriological reactions.

Clostridium welchii (*Bacillus welchii*): This organism is found rather widely distributed in nature. It seems to be able to live both saprophytically and parasitically. Koser reported its presence in a starter vended for making self-rising bread. During the World War, it was the most important organism in wounds. Evidently it may play a double role, being pathogenic at times and non-pathogenic at others.

Morphology. Short, thick, stubby rods; short chains of 2 or 3 rods may be formed; capsules frequently seen; non-motile.

Staining reactions. Gram-positive.

Sporulation. Spores formed but not abundant.

Blood agar. Dew-drop in form, hemolytic, transparent at first, later becoming opaque in two to three days.

Gelatin. Actively liquefied and blackened.

Fermentation reactions. Ferments glucose, sucrose and lactose with acid and gas.

Pathogenicity. Pathogenic for laboratory animals.

Habitat. Water, soil, infected wounds, etc.

Clostridium tetani (*Barillus tetanus*): The disease caused by this organism will be described in a later chapter. It is one of the oldest anaerobic spore-forming bacteria. The organism has the following characteristics:

Morphology. Slender rods, $0.5-0.8 \times 2-4$ microns; often grow in long filaments; motile.

Staining reactions. Gram-positive; easily stained by ordinary aniline dyes.

Oxygen relations. Anaerobic.

Sporulation. Resistant spores formed, occurring in the ends of the rods.

Gelatin. Slow growth on gelatin plates; colonies are formed with dense, opaque centers; slow liquefaction of the gelatin.

Agar. Colonies are hairy and fine.

Pathogenicity. Pathogenic for many animals.

The Coccaceae.—Into this group are placed most of the round bacteria irrespective of the space arrangement of the cell masses. Hucker in 1924, published a comprehensive study of these bacteria, finding that *Micrococci*, *Staphylococci* and *Rhodococci* could be placed in one genus for which he retained the name, *Micrococci*. These organisms were characterized as follows:

Micrococcus (Cohn, 1872): Cell division in two or three planes. Generally does not produce regular tetrads or packets. Typically saprophytic or a facultative parasite. May or may not produce red, yellow, or orange pigment. Produce moderate to abundant growth on ordinary nutrient media. Gram-

positive, variable, or negative. Aerobic or a facultative anaerobe. Does not produce indol. Gelatin often liquefied. Rarely attacks starch. True endospores have not been found. Nitrates often reduced. Motile species have been described. Produces acid but rarely gas from the fermentation of sugars.

After studying a great number of coccus forms, Hucker prepared the following key:

The term *Micrococcus* has been employed to designate the genus of irregular mass-forming cocci and, after a study of a long series of cultures, 16 species have been found which appear to be more or less constant. Gelatin liquefaction, nitrate reduction, and ability to utilize certain ammonium salts as the only source of nitrogen have served as the most important basis for the differentiation of these species. The species of this genus may be outlined as follows:

KEY TO THE SPECIES OF THE GENUS MICROCOCCUS

A. Pigment produced.

I. Yellow pigment produced.

1. Gelatin liquefied.

a. Nitrates reduced.

* Utilizes $\text{NH}_4\text{H}_2\text{PO}_4$ as a source of nitrogen.

M. conglomeratus

** Does not utilize $\text{NH}_4\text{H}_2\text{PO}_4$ as a source of nitrogen.

M. citreus

b. Nitrates not reduced.

M. flavus

2. Gelatin not liquefied.

a. Nitrates reduced.

M. varians

b. Nitrates not reduced.

M. luteus

II. Red pigment produced.

1. Gelatin liquefied.

M. roseus

2. Gelatin not liquefied.

M. cinnebareus

III. Orange pigment produced.

1. Gelatin liquefied.

M. aureus

2. Gelatin not liquefied.

M. aurantiacus

B. No pigment produced.

I. Cells in irregular masses.

1. Gelatin liquefied.

a. Nitrates reduced.

* Utilizes $\text{NH}_4\text{H}_2\text{PO}_4$ as a source of nitrogen. Acid proteolytic action upon milk.

M. caesi

** Does not utilize $\text{NH}_4\text{H}_2\text{PO}_4$ as a source of nitrogen.

M. albus

b. Nitrates not reduced.

* Utilizes urea as a source of nitrogen.

M. ureae

** Does not utilize urea as a source of nitrogen.

M. freudenreichii

2. Gelatin not liquefied.

a. Nitrates reduced.

M. epidermidis

b. Nitrates not reduced.

M. candidus

II. Cells in definite tetrads.

M. tetragenus

The following descriptions were reported by Hucker for the species studied.

1. *Micrococcus luteus* (Schroeter 1872) Cohn, 1872: Saprophytic. Abundant yellow growth on agar slant. Gelatin not liquefied. Nitrates not reduced. Generally inert in milk or produce acid not sufficient to curdle. Can utilize ammonium salts as the only source of nitrogen. Generally Gram-negative. Commonly found in milk or milk products. Cells occur in large groups. Synonym: *Bacteridium luteum* Schroeter, 1872.

2. *Micrococcus varians* Migula, 1900: Saprophytic. Abundant yellow growth on agar slant. Gelatin not liquefied. Nitrates reduced. Can utilize ammonium salts as the only source of nitrogen. Generally Gram-negative. Variable in action upon milk. Found under a variety of saprophytic conditions. Occurs in large masses. Generally ferments dextrose without the formation of gas.

3. *Micrococcus flavus* Lehmann and Neumann, 1896: Saprophytic or parasitic. Liquefies gelatin. Does not reduce nitrates. Generally does not ferment mannite or glycerin. Gram variable. Forms large clumps of cells. Generally produces growth when ammonium salts are furnished as the only source of nitrogen. Produces an abundant yellow growth on agar slant.

4. *Micrococcus conglomeratus* Migula, 1900: Forms large clumps of organisms, many times in pairs. Liquefies gelatin. Varies in acid production in milk, but

generally does not produce sufficient acid to curdle. Usually does not ferment mannite or glycerin. Can utilize ammonium salts as the only source of nitrogen. Reduces nitrates. Produces an abundant light yellow growth on agar slants. Gram variable.

5. *Micrococcus citreus* Migula, 1900: Parasitic. Moderate yellow growth on agar slant. Grows slowly at all temperatures. Generally does not ferment glycerin or mannite. Produces acid coagulation of milk with slight expression of whey. Forms large clumps on laboratory media, but may show short chains or small clumps when freshly isolated. Liquefies gelatin. Reduces nitrates. Gram variable, usually positive. Will not grow when ammonium salts are furnished as the only source of nitrogen.

6. *Micrococcus aureus* (Rosenbach, 1884) Migula, 1900: Parasitic. Moderate growth on agar slant, becoming orange. Liquefies gelatin. Generally reduces nitrates. Usually Gram-positive. Generally ferments lactose and dextrose with the production of acid but no gas. Occurs in pairs and short chains when freshly isolated, but in large clumps when examined from older cultures. Will not utilize ammonium salts as the only source of nitrogen. This organism, usually called *Staphylococcus aureus*, is found in pus present in many inflammatory infections. It is also widespread in other places.

7. *Micrococcus aurantiacus* (Schroeter, 1872), Cohn, 1872: Saprophytic or parasitic. Moderate growth on agar slant with the production of an orange pigment. Gram variable, generally positive. Does not liquefy gelatin or liquefies very slowly. Generally reduces nitrates. Does not utilize ammonium salts when furnished as the only source of nitrogen. Forms short chains or large clumps. Synonyms: *Bacteridium aurantiacum* Schroeter, 1872.

8. *Micrococcus albus* (Rosenbach, 1884), Buchanan, 1911: Parasitic. Moderate to abundant white growth on agar slant. Gram variable, usually positive. Forms short chains or large clumps. Liquefies gelatin. Reduces nitrates. Does not utilize ammonium salts as the only source of nitrogen. Generally ferments glycerin and mannite. Generally forms an acid curd in milk with the expression of a small amount of whey. This species is known better under the name *Staphylococcus albus*.

Micrococcus tetragenus Gaffky, 1883: Parasitic. Occurs in tetrads, especially when freshly isolated or from liquid culture. Does not liquefy gelatin. Nitrates not reduced. Moderate white growth on agar slant. Grows best at 37° C. Generally ferments lactose and dextrose. Will not utilize ammonium salts as the only source of nitrogen. Gram-positive.

Micrococcus roseus Flüge, 1886: Saprophytic. Cells in pairs or large clumps. Generally Gram-negative. Generally liquefies gelatin. Can utilize ammonium salts as the only source of nitrogen. Produces moderate to abundant red growth on agar slant. Generally does not curdle milk with acid curd. Variable in relation to nitrate reduction.

Micrococcus cinnebareus Flüge, 1886: Saprophytic. Produces moderate to abundant growth on agar slant. Will not utilize ammonium salts as the only source of nitrogen. Generally does not liquefy gelatin. Usually Gram variable. Generally curdles milk. Forms large irregular clumps of cells.

Diplococcus pneumoniae: This is the bacterium which causes lobar pneumonia. It was discovered in 1886 by Weichselbaum and since that time has been described under a number of different names. The disease which this organism causes is described in a later discussion. This organism is characterized in advanced textbooks and a description will not be included at this time.

The Streptococci.—The genus *Streptococcus* is fully characterized on page 99. A few of the more common species will be discussed here.

Streptococcus pyogenes: This is a disease-producing microorganism; it is a member of a group of streptococci which produce hemolysis on blood agar plates. The streptococci in this group are spoken of as hemolytic streptococci. *Streptococcus pyogenes* is found in many of the acute inflammatory infections of man and animals.

Streptococcus mastitidis: This species is the causal agent in mastitis, an inflammatory infection in the mammary glands.

Streptococcus lactis: This is the streptococcus especially active in the souring of milk. It has been described under several different names. The name *Streptococcus lacticus* has been widely used although *Streptococcus lactis* is the proper term since it was the first one applied to the organism. It is briefly described as a member of the lactic acid group. It is described below.

Streptococcus fecalis: This streptococcus was isolated from the feces of man and animal. It is probably not ordinarily pathogenic. It has been confused with other strains which have significance in disease. It has been suggested, at times, as an indicator of pollution to be used in place of or along with *Escherichia coli* (*Bacterium coli*).

Streptococcus scarlatinae: Several different streptococci have been reported as the etiological agents in scarlet fever. The most recent one was described by Dick and Dick. This organism is better known by its serological characters rather than by its cultural characteristics.

The Lactic Acid Group.—This group of bacteria includes species which form especially large amounts of lactic acid. Probably all species form a little, but bacteriologists have found it convenient to place those which form larger amounts in one group. Members of this group are especially important in the dairy industry where they bring about souring of the milk or are used for the development of desirable flavors. Only a few of the more important species will be described.

Streptococcus lactis: This organism is supposed to be the most active one in the souring of milk. The name has been a matter of controversy and it is probable that it is also known under such names as *Bacterium lactis acidii*, Leichmann, *Streptococcus lacticus*, Kruse, and other names. Since Lister seems to have been the first to describe it and since he proposed the name *Streptococcus lactis*, that name should be used.

Morphology. Round cells with some cells coccoid in shape.

Staining reactions. Gram-positive.

Sporulation. No spores formed.

Agar media. Small colonies with entire edge.

Litmus milk. Coagulation with reduction of the litmus. The cultures usually

show complete reduction with white color in the lower portions of the culture tube and a narrow red ring at the top.

Fermentation reactions. No gas formed in common sugars. Acid formed in common sugars such as glucose, fructose, galactose, lactose, maltose, etc.

Habitat. Widely distributed; commonly present in milk.

Pathogenicity. Ordinarily not pathogenic.

Lactobacillus acidophilus: This species has been of considerable interest in the preparation of acidophilus milk which is described elsewhere in this book. It is one of the so-called acidophilic or aciduric bacteria in that it likes or forms considerable amounts of acid from sugars.

Morphology. Long slender rods, frequently granular.

Staining reactions. Young cells are usually Gram-positive, but old cells are often Gram-negative.

Agar media. Moderate growth; translucent.

Litmus milk. Coagulum with acid formation.

Fermentation reactions. Acid formed in common sugars; no gas formation.

Habitat. Intestinal canal of animals.

Pathogenicity. Non-pathogenic.

The Typhoid-Paratyphoid Group.—This group contains many species important in disease and sanitary bacteriology. For convenience, the group is subdivided according to salient characteristics of the several members. Many of the members of the typhoid-paratyphoid group are intestinal forms having a permanent abode in the intestinal tracts of man and other animals. Some of them cause severe intestinal infections. The more important species of significance in disease are *Eberthella typhi* (*Bacterium typhosum*); *Salmonella paratyphi* (*Bacterium paratyphosum A*); (*Bacterium paratyphosum B*) *Salmonella schottmulleri*; (*Bacillus enteritidis*) *Salmonella enteritidis*; etc. (*Bacterium typhosum*) *Eberthella typhi* probably causes the most severe infection.

The term paratyphoid was introduced for some bacterial species which were much like *Eberthella typhi* both in characteristics as well as the type of infection caused in human beings. The term "Salmonella group" has also been used for a sub-group, the organisms in which are especially significant in food poisonings and infection.

The Dysentery Organisms.—These organisms have been proven to cause epidemic dysentery. The organism is present in great numbers in the stools from infected individuals. The organism probably does not get into the blood stream but remains localized in the mucosa of the intestines. Several types of varieties of the species are known. They possess many of gross characteristics in common but are separable by means of the more delicate serological reactions. The organisms in this group are not of so much interest in elementary courses.

The Colon-Aerogenes Organisms.—The Colon-Aerogenes organisms are of much significance in sanitary bacteriology. They are present in the alimentary tracts of man and the lower animals. The members of this sub-group are generally non-pathogenic, although if the conditions are right they may cause disease. *Escherichia coli* (*Bacterium coli*) has been isolated from cases of cystitis, meningitis, peritonitis, etc. It may also be a plant pathogen under some conditions.

Escherichia coli (*Bacterium coli*, *Bacillus colon*, etc.): This organism has been discussed also in the chapter on Water Bacteriology, a field of applied bacteriology in which it enjoys a well-established role as an indicator of pollution. At this time only its important characteristics will be enumerated.

Morphology. Short rods (often coccoid) 0.5 by 2.0–4.0 microns; motile in most cultures; peritrichic flagella.

Staining reactions. Gram-negative; stains easily with the common staining solutions.

Plain broth. Good growth, uniform turbidity.

Plain gelatin. Thin moderate growth; no liquefaction.

Agar. Abundant, opaque growth.

Milk. Acid coagulation.

Fermentation reactions. An active fermenter; acid and gas formed from glucose, lactose, mannite dulcite, sorbite.

Voges-Proskauer reaction. Negative (no acetyl-methyl-carbinol formed).

Methyl red reaction. Positive.

The characteristics for differentiating this organism from its very near relative are tabulated in Chapter XX.

Aerobacter aerogenes (*Bacillus aerogenes*, *Bacillus lactis aerogenes*, etc.): This organism was formerly confused with *Escherichia coli*, but methods have been devised for its separation from this organism. These have been tabulated in Chapter XX. It was believed in 1885 to be the organism which caused souring of milk. Later work, however, showed that it was not important in this connection. The organism is now chiefly of interest on account of its significance in sanitation and the fact that it may be easily confused with *Escherichia coli* (*Bacterium coli*). These facts are discussed in the chapter on Water Bacteriology.

Morphology. Very much like *Escherichia coli* (*Bacterium coli*); non-motile; may at times form capsules.

Staining reactions. Stains easily with ordinary staining solutions.

Gelatin. No liquefaction. Good growth. White opaque.

Agar. Good growth.

Fermentation reactions. Gas formed in dextrose, sucrose, lactose, levulose, maltose, galactose and arabinose. Forms twice as much carbon dioxide as hydrogen.

Oxygen relations. A strict aerobe.

The Proteus Group.—The organisms of this group were described as follows in Bergey's Manual of Determinative Bacteriology: "Pleomorphic rods. Filamentous are curved rods and common as involutions forms. Gram-negative. Generally actively motile, possessing peritrichous flagella. Produce characteristic amoeboid colonies on moist media and decompose proteins. Ferment dextrose and sucrose but not lactose. Do not produce acetyl methyl carbinol. The type species is *Porteus vulgaris* Hauser."

Bengtson described the organism as follows:

"A Gram-negative rod, which may exhibit pleomorphism; non-spore-forming, motile, non-capsulated, peritrichic flagella, liquefying gelatin; characteristic colonies often formed; acid and gas formed from dextrose; saccharose, maltose, and mannite often fermented; lactose never fermented; casein precipitated then dissolved; putrefactive odor formed in protein compounds; indol usually formed; nitrates reduced; hydrogen sulfide formed; growth on agar rapid and spreading; brown pigment production in broth; Voges-Proskauer reaction negative."

Other species of the Proteus group are:

Proteus mirabilis

Proteus hydrophilus

Proteus asiaticus

Proteus valeriae

Chromogenic Group.—The chromogenic organisms are those which produce pigments. The pigment may remain in the cell or diffuse out of the cell into the medium. The latter is, at times, a fluorescent pigment. The best-known chromogenic bacteria are, perhaps, *Serratia marcescens* (*Bacillus prodigiosus*) and *Chromobacterium violaceum* (*Bacillus violaceus*).

Chromobacterium violaceum (*Bacillus violaceus*): This species is a common saprophyte in nature, being readily isolated from soil and water. It seems to form the pigment as an excretory product but retains it within the cells.

Morphology. Rods 0.1–4.0 microns; single or in chains; motile by means of peritrichic flagella.

Staining reactions. Gram-negative; easily stained by ordinary staining solutions.

Gelatin. Rapid liquefaction; colonies are round with entire margin; contain areas with dark blue or violet coloration.

Agar. Colonies white, moist with violet areas. Abundant growth.

Broth. Moderate growth; ring with violet coloration.

Milk. Peptonization; alkaline reaction.

Oxygen relations. Aerobic.

Habitat. Water, soil, etc.

Serratia marcescens (*Bacillus prodigiosus*): No organism in bacteriology has a more striking and interesting history than this one. Certain authors make

numerous allusions to the appearance of "bloody bread." Some of the instances were connected with bread used at the Sacrament.⁴ The belief that blood had appeared on the bread is easily understood to-day.

Morphology. Rods 0.5×1.0 micron; isolated cells or in chains; motile by means of several peritrichic flagella.

Gelatin. Active liquefaction with appearance of red coloration; with some strains deeply colored. Colonies are thin with undulated edge; colored red.

Agar. Round, granular; become red with age. Rapid growth on slants; better pigment formation at temperatures below 37°C .

Broth. Abundant growth with red ring about meniscus line.

Milk. Coagulation, acid; very slow peptonization.

Oxygen relations. Aerobic.

Fermentation reactions. Inactive.

Pathogenicity. Non-pathogenic.

Habitat. Soil, water, etc.

The Fluorescent Group.—The members of this group are characterized by the ability to form a fluorescent pigment. This pigment differs somewhat from other pigments in that it is diffusible into the surrounding medium giving, with some cultures, a beautifully colored medium. The members of the group make up a fairly homogeneous group in that most of them are non-spore formers. They are separated into subgroups by gelatin liquefaction, formation of hydrogen sulfide and some of the fermentation tests.

The classification by the Committee of the Society of American Bacteriologists groups many of these forms as *Pseudomonas*, the characteristics of which are given on page 88.

Pseudomonas fluorescens: This is one of the commonest species encountered in soil, water, sewage, etc. In the older literature two types were recognized depending on whether gelatin was liquefied or not. This species has the following characteristics:

Shape and size. Rods 0.5 to 1.0–2.0 microns; occurring singly or in chains of several units; polar flagella.

Staining reactions. Gram-negative; stains with the ordinary staining solutions.

Gelatin. Liquefaction in tubes and on plates; media colored green.

Agar. Good growth with green fluorescence.

Broth. Growth abundant with green coloration.

Milk. No coagulation; alkali formed with slow peptonization.

Oxygen. Aerobic.

Fermentation. Inactive.

Pseudomonas aeruginosa (*Bacillus* or *Pseudomonas pyocyaneus*): This species is pathogenic. It has received much study.

⁴ Harrison, F. C. The "Miraculous" Micro-Organism. Trans. Roy. Soc. Canada, Section V, Series III, 18 (1924).

Morphology. Rods often in chains; 0.5–2.0 microns; polar flagella; motile.

Staining reactions. Gram-negative; stains easily by ordinary aniline dyes.

Gelatin. Rapid liquefaction; medium colored green. Colonies on gelatin plates, hairy, with uneven edge.

Agar. Abundant growth the medium being colored green; old cultures dark brown.

Broth. Abundant growth; pellicle; marked sediment.

Milk. Coagulation, peptonization.

Fermentation reactions. Inactive; no gas in sugars.

Oxygen relations. Aerobic.

Pathogenicity. Pathogenic for common animals.

Acid-fast Group.—This group is created on the basis of reaction to a certain method of staining. Once the cells have been stained, they are resistant to the action of rather strong acid and are thus said to be “acid-fast.”

The Tubercle Bacilli: The microorganisms causing tuberculosis first isolated by Koch are the best-known members of the acid-fast group. The characteristics are so easily accessible in reference texts that they will not be reproduced here. The tuberculosis bacteria are separated into four types depending on the host for which each is more susceptible. Some information exists to show that all of these forms constitute one type of microorganism irrespective of the host which is infected. This is still a controversial question and queer results have been secured when attempts have been made to change one type into another. Bergey and the committee which helped prepare his Manual of Determinative Bacteriology proposed the following names for four members of the tuberculosis group:

(*Mycobacterium tuberculosis* [hominis]: This type has special affinity for human tissue. It is well known as the cause of pulmonary tuberculosis but may be isolated from other lesions or foci of infection in the human body.

Mycobacterium tuberculosis [bovis]: The bovine type of the tuberculosis organism is present in tuberculous cattle. It causes most of the tuberculosis in children but less of that in adult human beings. The bovine type is more virulent for animals than is the human type.

Mycobacterium avium: This type causes tuberculosis in fowls and birds. It does not infect guinea pigs easily while certain other laboratory animals such as mice and rabbits are susceptible.

Mycobacterium piscium: The so-called fish type causes tuberculosis in fish, turtles and other cold-blooded animals. This type is rather inactive in warm-blooded animals.

Mycobacterium smegmatis (*Bacillus smegma*): This acid-fast organism is of significance only in that it may cause confusion in laboratory diagnosis of tuberculosis. It is present on the genitals of man. At one time it was said to be the cause of syphilis; further experimental work, however, proved this to be incorrect.

Mycobacterium leprae: This organism was isolated by Hansen in 1873 as the cause of leprosy. It seems to cause disease only in man since the lower animals have never been infected. The experimental work in leprosy constitutes a very interesting chapter in medical science. The organism is fully described in advanced texts in medical bacteriology.

The Thermophilic Group.—The thermophilic group is made up of bacteria with temperature relations markedly higher than ordinary bacteria. They are, in general, spore-forming and tend to be aerobic. Several anaerobic species have, however, been described. They multiply very rapidly at 55° C., giving at this temperature in five or six hours a large colony on agar. They are rods of varying length. They are not active fermenters but a few species decompose cellulose with the formation of products of commercial importance among which are acetic acid, alcohol, etc.

Morphology. Rods, usually in chains motile.

Staining reactions. Gram-positive; stain readily with ordinary staining solutions.

Sporulation. Central or polar spores formed. In some cases spore may be larger than the cell.

Broth. Abundant growth at 55° C.; sediment; heavy pellicle.

Agar. Abundant growth.

Gelatin. Liquefaction.

Hydrogen sulfide. Hydrogen sulfide formed from peptone.

Milk. Peptonization and coagulation.

Fermentation reactions. No gas formation; acid formed in some cases.

Oxygen relations. Aerobic.

Temperature relations. Optimum 55°–60° C.; minimum 40° C.; maximum 82° C.

Habitat. Water, soil, etc.

Pathogenicity. Non-pathogenic.

Diphtheria Group.—Under this heading are grouped several different species of bacteria having morphological and physiological characteristics in common. The group is easily separated into subgroups on the basis of pathogenicity.

Corynebacterium diphtheriae (*Bacillus diphtheriae*): This organism was isolated in 1863 by Klebs. It occurs in the membranes or exudates in the throats of persons suffering from diphtheria. It is also known to cause other lesions. The organism is quite pleomorphic, being known in a number of types. Under usual conditions, it stains with a granular structure. The disease caused by this organism is discussed in Chapter XXVI.

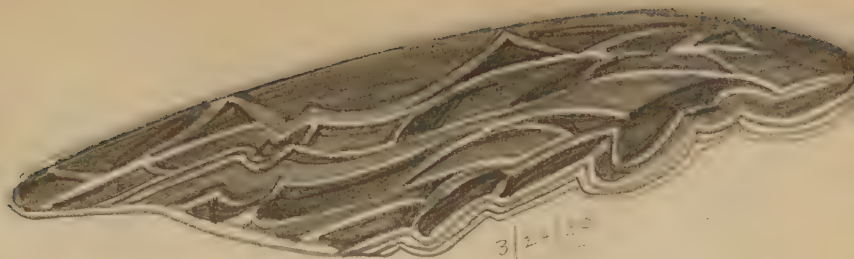
Corynebacterium hoffmani (*Bacillus Hoffmani*): This bacterium is called by many authors the pseudo-diphtheria bacillus or Hoffmann's bacillus. This organism is much like *Corynebacterium diphtheriae* but does not produce a toxin nor ferment carbohydrates in the same manner.

The Vibrio Group—Spirillum Forms.—Perhaps the best-known member of this group is *Vibrio comma* (*Microspira cholerae*), the cause of cholera. The microorganism has been subjected to considerable study and is described in texts on pathogenic bacteria.

A spirillum form is concerned with the reduction of sulfates in the sulfur cycle. Two species are known to reduce sulfates, *Spirillum desulfuricans* and *Spirillum aestuarii*.

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CHAPTER VII

MOLDS

Molds are distinguished from the yeasts and bacteria by being multicellular organisms. Consequently their morphology and physiology are more complicated but also more interesting. Macroscopically, the vegetation of a mold resembles a bit of cotton and is called the *mycelium*. Each thread or filament of this mycelium is called a mycelial thread or hypha (*plu.*, hyphae). Two kinds of hyphae are formed depending on the purpose for which they exist. Fertile hyphae are those which bear the fruiting bodies—reproductive organs. Some of these are represented later for the more common genera. Vegetative hyphae are those which are concerned in the nutrition of the mold plant. They are comparable, therefore, to the vegetative or actively growing form in the bacteria and other fungi. Mycelial threads are also distinguished from one another by the presence or absence of cross-walls or septa (*sing.*, septum). Those threads which have cross-walls are said to be septate; those without cross-walls, non-septate or coenocytic. This, then, is one differential characteristic by which molds may be subdivided.

Hyphae may be submerged or aerial. *Submerged* hyphae extend down into the medium on which the mold may be growing; *aerial* hyphae extend up into the air. The aerial hyphae probably function in securing oxygen, getting rid of excess moisture, etc. The submerged hyphae anchor the mold more solidly so that it may produce an abundant aerial vegetation.

Structure of Hyphae.—Each mycelial thread of a typical mold is made up of numerous cells each of which has the various organs found in all typical cells. The cell wall is composed of a resistant substance the chemical nature of which is not generally agreed upon. Some investigators have reported chitin while others have called the structural substance fungus cellulose.

Reproduction of Molds.—Reproduction among the molds is accomplished by special units called spores or conidia (*sing.*, conidium). The characteristic color of the mold is due to the pigments contained in the conidium. Each conidium is able to reproduce the species and

is therefore comparable, perhaps, to the seeds of the higher plants. Great numbers of conidia are formed by each mold plant and it is easy to understand how molds are so prolific. The hyphae which bear the conidia and the organs forming them are called conidiophores. This process of reproduction is often spoken of as fructification by conidia. The conidia are formed at the tips of certain mycelial threads. They are.

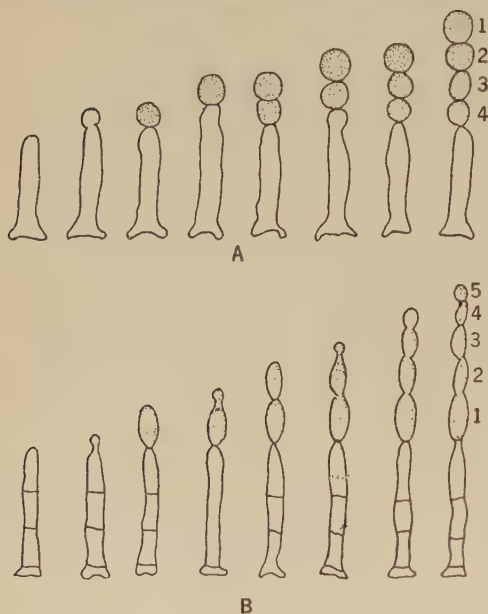


FIG. 47.—Diagrammatic Illustrations of Methods of Conidial Fructification. (After Zopf.)

A. The conidium is formed first from an enlargement at the tip of the mycelium. Repetition of this process gives rise to the rest of the conidia.

B. In this case the conidiophore constricts to form the first spore. Each succeeding spore is formed from a preceding one. They are formed in just the reverse order as in A.

the oldest conidium or spore may not be at the tip of the chain but next to the mycelium.

Another method of spore production may be spoken of as fructification by sporangia. With this method the spores or conidia are borne within a membrane called a sporangium. This is the case with *Rhizopus nigricans*.

Asexual Spores.—As the mold plant which develops from a spore grows and becomes older, certain changes may be observed at the tips of certain of the mycelial threads depending on the species. These

in many cases, pushed out into a chain, the oldest conidium, of course, being at the end of the chain away from the tip of the hypha.

Two methods of conidium formation have been described. They are illustrated in Fig. 47. The first method (shown in A) begins with the formation of an enlargement which becomes round and finally is surrounded by a membrane. The first conidium is thus formed. The process is then repeated and thus the oldest conidium is at the tip of the chain.

The other method (shown in B) is different. After the first conidium is formed, this one forms a little bud or projection which develops into the next spore. In this case

threads bear the so-called fruiting bodies or organs which are to form the conidia or spores, as described in the preceding paragraph.

Some molds produce asexual spores without the formation of special organs. The whole mycelium seems to be able to break up into segments called oidia (*sing.*, odium). Such cells are possessed of the properties of spores or resting cells.

Sexual Spores.—

This heading implies the formation of a spore by the union of two cells, spoken of as

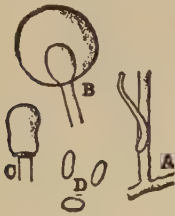


FIG 48.—*Mucor Mucedo*.
(After Jensen.)

A, Part of a Sporangiophore Showing Occasional Branching; B, Sporangium; C, Columella; D, Spores.

gametes. The fusion of two gametes to form a *zygospore* is nicely shown in Fig. 49.

Mucormucedo. After fusion of the gametes, the cell wall thickens and the cell takes on the properties of a typical spore. Zygospore formation does not take

place in molds having septate mycelium. The zygospore persists until favorable conditions obtain when it germinates to reproduce the species. The gametes or cells about to fuse are often at the tips of two hyphae. After fusion, the zygospore or zygota may become free.

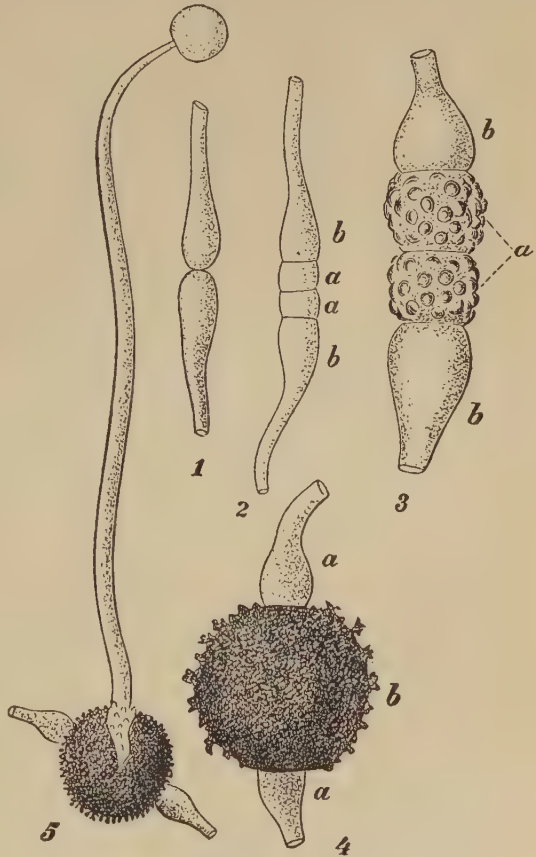


FIG. 49.—*Mucor mucedo*. Formation of the Zygospores.
(After Brefeld.)

1. Two hyphae in terminal contact. 2. Articulation into gamete *a* and suspensor *b*. 3. Fusion of the gametes *a*; the membrane thickens. 4. Ripe zygospore *b* supported by the suspensors *aa*. (Magn. of 1-4, 225.) 5. Germination of the zygospore to a sporangium stem. Magn. about 60.)

Properties of Mold Spores.—Since the mold spore or conidium is a resting stage in the life cycle of this plant, it is to be expected that it possesses certain powers of resistance. Pasteur showed that moist heat was much more efficient than dry heat. The spores of *Penicillium glaucum* were killed quickly by boiling but survived dry heat at 120° C. for an appreciable time. Mold spores also survive cold and treatment with disinfectants. They are far more resistant than ascospores of yeasts but, perhaps, not quite so resistant as spores of some bacteria. Data on the effect of the several physical agents on mold spores are not abundant in the literature. Another investigator by the name of Lode stated that dry heat of 125° C. killed all mold spores in fifteen minutes; 80° C. for seven hours was without effect. Weinzirl reported that mold spores withstood from fifty-eight hours to five days continuous exposure to sunlight.

Germination of Spores.—Spores are resting stages which are very resistant to unfavorable conditions. When favorable conditions are present the spore may germinate—develop into the active growing stage. This involves, of course, rupturing of the outer wall with often an extrusion of the inner wall. Finally a thread-like structure is formed and the plant is ready again to reproduce these steps.

MUCORS

This is one of the commonest genera of molds and also one of the most interesting on account of the industrial significance of certain of its members. The species included in the genus are well distributed in nature. They are very active in the destruction of organic matter, one species being used in the manufacture of ethyl alcohol by the so-called amylo-process.

General Morphology.—The general characteristics of the members of this group may be briefly stated as follows: there is a strong development of threads beneath the surface of the medium which support the aerial structures. These threads, comparable to the runners of strawberry plants, are called *stolons*. The aerial hyphae, possessing sporangia at their tips, are called sporangiophores. Within the sporangium are formed great numbers of black spores. When the sporangium breaks, these spores are released. The mycelium of the mucors is non-septate, giving us one useful characteristic with which to separate them from other molds.

KEY TO COMMON MUCORS AS PREPARED BY JENSEN, 1912

MUCOR

- I. Sporangiphores unbranched.....*Mono-mucor*
 II. Sporangiphores branched
 1. Branching rare or more frequent, and in this latter case indefinite, in clusters
 or in corymbs.....*Racemo-mucor*
 2. Branching definite, in sympodia.....*Cymo-mucor*

Mono-mucor

- Sporangiphores not branched (exception, when the nutrition is unfavorable,
 branching occurs; this, however, is anomalous).
 1. Sporangiphores not exceeding 2 cm. in length..... 2
 Sporangiphores more than 2 cm. long..... 3
 2. Sporangia red, 20–35 μ , with diffuent membrane, spores 2.5 by 2 μ .
 M. Ramannianus Möller
 Sporangia green-black, 50–80 μ usually 60 μ , with diffuent membrane, spores 4–10
 by 2.5–5 μ*M. hiemalis* Wehmer
 Sporangia brownish, 30–80 μ mostly 60 μ , with nondiffuent membrane, spores
 2–3 by 1.5 μ*M. microsporus* Namyłowski
 Sporangia gray, 80–95 μ , with diffuent membrane, spores 8.5 by 4.5 μ .
 M. adventitius Oudemans
 3. Sporangiphores 1–4 cm. long, spores most cylindrical, 5–7 by 2.5–3.5 μ
 M. strictus Hagem
 Sporangiphores 2–15 cm. long, spores 6–12 by 3–6 μ
 M. mucedo (Linn?) Brefeld

Racemo-mucor

- Branching indefinite, in groups or clusters or in corymbs.
 1. Sporangiphores at first erect, but soon falling..... 2
 Sporangiphores always erect..... 3
 2. Branches of the sporangiphores ascending; sporangia 40–60 μ , bright yellow,
 breaking, leaving a basal collar.....*M. Christianiense* Hagem
 Branches of the sporangiphores mostly reflexed; sporangia 15–45 μ , falling closed
 and remaining intact for a long time.....*M. dispersus* Hagem
 3. Spores very unequal (mixture of numerous small spores with others twice as large),
 oval or subcylindrical, 4 by 2 μ or 5 by 3 μ to maximum 8 by 6 μ
 M. sylvaticus Hagem
 Spores reasonably equal..... 4
 4. Membrane of the sporangium nondiffuent.....*M. racemosus* Fresenius
 Membrane of the sporangium diffuent..... 5
 5. Species very large, 6–8 cm. high (always over 2 cm.); spores 9–12 by 4.2 μ
 M. flavus Bainier
 Species much smaller, never exceeding 2 cm. high; spores 5–7 by 2.5–3.5 μ
 M. genevensis Lendner

Cymo-mucor

Sporangiophores branched in sympodial cymes.

1. Sporangioophores nonerect, incurved, forming a cobwebby network, 1.5 cm. high, ending in a large sporangium and producing at a short distance below more or less closely clustered branches; secondary branches sometimes dichotomous. 2
Sporangiophores circinate. 3
Sporangiophores straight, not circinate. 6
2. Spores average 8μ *M. botryoides* Lendner
Spores average 6μ *M. botryoides* var. *minor*
3. Sporangioophores never much surpassing 1 cm.; spores oval, 6μ being maximum length. 4
Sporangiophores 0.5–2 cm. long, the longer noncircinate, the shorter much branched and circinate, sporangia average 60μ , spores average 10μ
M. lamprosporus Lendner
4. Membrane of the sporangium brown; sporangia often subsessile, spores oval or broad elliptical, 3–4 by $5-6\mu$ *M. circinelloides* van Tieghem
Sporangia supported by very long pedicels, often circinate 5
5. Sporangia $60-80\mu$ in diameter, spores 4–6 by 4μ *M. griseo-cyanus* Hagem
Sporangia $50-60\mu$ in diameter, spores 5–7 by $3.5-5\mu$ *M. corticolus* Hagem
6. Spores globose. 7
Spores oval. 10
7. Species grows poorly on wort-gelatin, forms on bread a short aerial growth of 2–3 mm. high sporangia $50-70\mu$ in diameter, spores globose, $5-6\mu$
M. Jansseni Lendner
Species grows well on wort-gelatin and forms an aerial growth 1–3 cm. high. 8
8. Columella spinescent. *M. plumbeus* Bonorden
Columella smooth. 9
9. Sporangia $70-110\mu$ in diameter, sporangioles not caducous, spores globose, brilliant, 10μ *M. sphacrosporus* Hagem
Sporangia never exceeding 80 to 90μ , spores 10μ , sporangioles circinate, caducous, sporangiophores more elevated than with *M. sphacrosporus*.
M. lamprosporus Lendner
Sporangia $60-80\mu$, spores normally $8-10\mu$, spherical or accompanied by abnormal oval spores $8-10$ by 30μ , no sporangioles. *M. dimorphophorus* Lendner
10. Sporangia $50-350\mu$, columella globose, spores globose or ellipsoidal or angular, $4.2-6.5\mu$, chlamydospores. *M. geophilus* Oudemans
Sporangia variable, lead-gray, those of the shorter sporangiophores $45-180\mu$, columella oval or ovate, spores broad ellipsoidal, $4.5-10$ by $3.5-7\mu$ usually 6.8 by 4.6μ *M. saturninus* Hagem

Fruiting Bodies.—The fruiting bodies of *Mucors* are quite different from those of other molds. They are borne on a non-septate mycelial thread, called a *sporangiophore*. At the tip of the sporangiophore is an organ called the *columella*; this has different shapes depending on the species of mold which is being studied. About this columella are formed large numbers of spores or conidia; these are in turn surrounded by a membrane called the *sporangium*. The sporangiophore which bears the fruiting bodies, arises singly from the stolons in the *Mucors*.

Great numbers of spores are liberated by each sporangium. These are disseminated and germinate to reproduce the species when they fall on good soil or media. Such spores are asexual.

Another type of spore, a sexual spore already discussed as a zygo-spore, is also formed by *Mucors*. Such a spore is shown in Fig. 49 *Mucor mucedo*.

Mucor mucedo was first described by Brefeld. It was characterized by Jensen (1912) as follows:

Mucor mucedo.—Sporangiophores erect, with colorless membrane and colorless or weak-yellow scanty contents, forming a thick silver-gray glistening sward over the entire substratum, usually unbranched, 2–15 cm. long by 30–40 μ thick, non-septate, occasionally, especially the first from young mycelium, much shorter and with a side branch; sporangia large, globose, 100–200 μ in diameter, at first yellowish, later when dry becoming dark gray or black-brown, occasionally with greenish shimmer, thick fine-spiny; sporangial wall quickly dissolving, leaving generally a basal collar; columella high-cylindrical or bell-shaped or globose, 70–140 μ high by 50–80 μ broad, with smooth, colorless membrane and mostly with orange-yellow contents; spores round cylindrical to long ellipsoidal, twice as long as broad, very uniform in shape, variable in size 6–12 by 3–6 μ (extreme forms 16.8 μ long), with colorless smooth membrane, weak-yellow or colorless contents.

Heterothallic; zygospores globose, 90–250 μ in diameter; exospore black, with thick projecting tubercles; endospore colorless; contents colorless, with large oil drop; zygospores in our cultures not observed.

Mucor rouxii.—This mold has been found in material known as Chinese yeast used in the preparation of fermented beverages prepared from rice. This species and some of its near relatives produce a very active amylase which has given the mold a place in several industrial fermentations. On account of its diastasic activity, *Mucor rouxii* was used in the so-called amylo-process for the preparation of alcohol from starchy materials. These materials, cornstarch, etc., are subjected to malt action and sterilized. Finally they are fermented with a culture of this mold. The spores germinate and the entire mass becomes permeated with the mycelium. The mold hydrolyzes the starch to fermentable sugars which are in turn fermented by yeast. In this case the mold accomplishes more efficiently what malt has been used for.

Rhizopus nigricans.—This is the best-known species of *Rhizopus* molds. It is widely disseminated and is usually the black mold appearing on starchy foods, such as old bread, kept in a moist place. It has also been found to be the cause of great losses in the shipment of fresh strawberries and other fruits. It was characterized as follows by Jensen (1912):

Rhizopus nigricans.—Stolons far-spreading, even growing onto and spreading over the culture glass, covering the substratum and its neighborhood with thick cobwebby

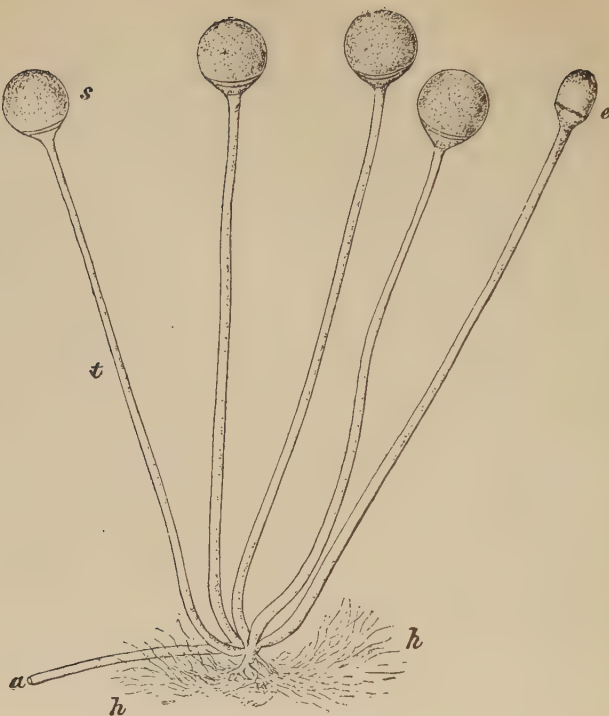


FIG. 50.—*Rhizopus nigricans* Ehrenberg. (After Brefeld.)

(a) Is the extremity of a stolon, which has developed into the appressorium (h). This latter is the starting point of the sporangiophores (t), four of which are shown with the sporangia (s) unbroken, whilst the columella (e) is all that remains of the fifth. Magn. 30.

growths 1–3 cm. long and occasionally still longer, simple or sparsely branched, occasionally fork-like internodes, with smooth, at first hyaline then finally brown, membrane,



FIG. 51.—*Rhizopus nigricans*. (After Jensen, 1912.)

A. Columella with adhering spores and apophysis. B. Columella after becoming pileate. C. Spores.

contents colorless; rhizoids more or less richly branched, at first with colorless, later with brown or black-brown smooth, thick membrane, as thick as 16μ at base and 5μ at tip, finally uniseptate; sporangiophores seldom single, mostly in clusters of 3–5 occasionally up to 10, unbranched, 0.5–4 mm. high, $24\text{--}42\mu$ thick, with smooth, finally brown or black-brown, membrane, contents colorless, with apophysis of sporangium more or less hemispherical, large, $100\text{--}350\mu$ broad, at first snow-white then becoming at maturity black, erect; columella with broad base. very large, broadly half globose, highly arched, with apophysis 70μ broad by 90μ high to 250μ broad by 320μ high, with brown smooth membrane, often covered with spores after opening of sporangia, often becoming pileate, spores irregularly globose or broad oval, mostly with one or two blunt corners, variable in size, $6\text{--}17\mu$ in diameter, mostly somewhat longer than broad, with thick double membrane on which are seen meridian-like folds, light pale gray, contents colorless; zygospores globose or

barrel-shaped $160\text{--}220\mu$ in diameter; exospore firm, brown-black, opaque, closely beset with half-, globose high warts; endospore colorless, thick, with small outgrowths filling the warts of the exospore; suspensors swollen, commonly unequal, almost as broad as the zygospore; azygospore observed.

THAMNIDIUM

This group is much like the Mucors. The members possess sporangiophores with a sporangium on the end, and also show a branching of the sporangiophore. At the tips of these branches are also found sporangia filled with spores. The members of this group are frequently found in foods.

Thamnidium elegans.—Sporangiophores erect, ending in large sporangia. Below these, in the middle or under part of the sporangiophore are generally two to five verticillate horizontal side branches that are forked several times (two to ten) after an obtuse-angled fashion. The branchlets finally end in sporangioles. The whole sporangiophore is usually $0.5\text{--}3$ cm., occasionally 6 cm. high by $25\text{--}35\mu$ thick. The white floccose group of forked verticillate branches are relatively very small, $0.25\text{--}1$ mm. broad. The main verticillate branches up to the forking are $150\text{--}200\mu$ long by 8μ thick; the forks of the first order are $40\text{--}60\mu$ long; the forks of the last order are only $4\text{--}6\mu$ long by 2μ broad. The membrane of the sporangiophore and its branches is colorless and smooth; contents are also colorless. Branching of the sporangiophore is very variable (see variation below). Chief sporangia (haupt-sporangia) globose, large, $100\text{--}200\mu$ diameter, occasionally smaller, at maturity white with large ovate or bell-shaped, colorless, smooth columella; sporangioles globose, small, white, $8\text{--}16\mu$ in diameter, mostly 4-spored, varying, however, from 1 to 10; spores of haupt-sporangia and sporangioles similar and equally large (except in the one-spored sporangioles where they are globose, $8\text{--}16\mu$), ellipsoidal, $8\text{--}10\mu$ by $6\text{--}8\mu$, smooth, weak gray-brown. Zygospores are globose, black, with thick black warty exospore and yellowish endospore. Zygospores not observed in our cultures.

Variation of sporangiophores—the branching of the sporangiophores is very variable. In one extreme case the sporangiophore carries only the chief sporangium, when it resembles an ordinary Mucor; in the other case the chief sporangium is wanting, the sporangiophore ending then either with a crown of sporangioles or with a sterile tip. Occasionally the first side branch ends in a sporangium, again the number and order of the branching are manifold.

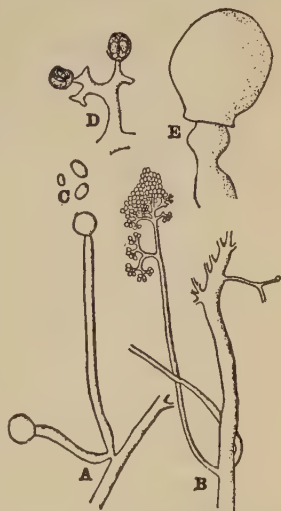


FIG. 52.—*Thamnidium elegans* Link. (After Jensen).

A. Sporangiophore and Haupt-sporangia; B. Sporangiophore and Nebensporangia; C. Ultimate Divisions of Sporangiophore with Sporangioles; D. Columella of Hauptsporangium.

PENICILLIUM MOLDS OR BLUE-GREEN MOLDS

These molds are common types in nature. They may be easily isolated from citrus fruits which have begun to spoil and show the presence of blue-green spots. The name penicillium comes from the Latin meaning little brush, because the gross form of the mold is brush

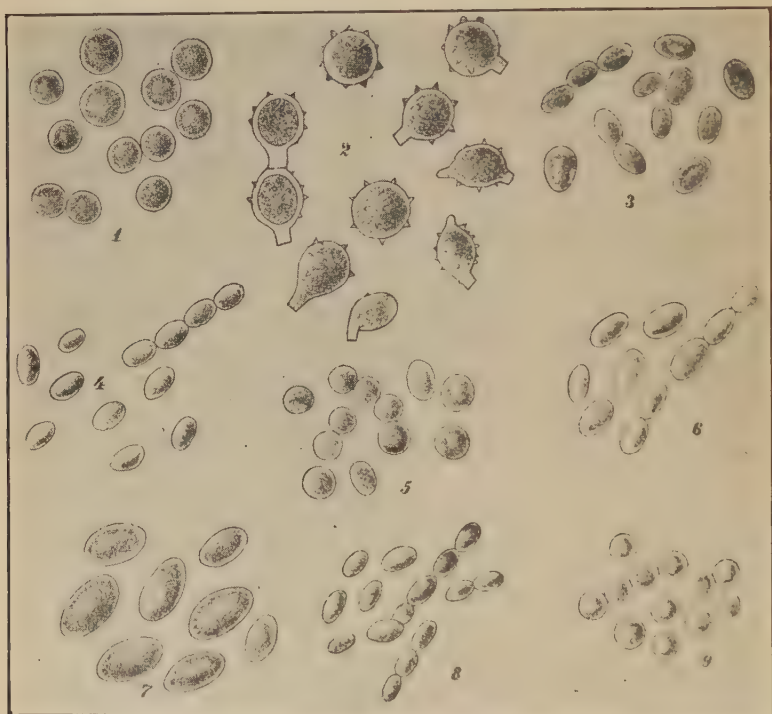


FIG. 53.—Conidia of Various Species of *Penicillium* Drawn to the Same Scale. (After Wehmer; in Lafar's *Handbuch der Technischen Mykologie*. By Permission of Gustav Fischer.)

1. *P. Camembert* (conidia $3.1\text{--}4.5\ \mu$ in diameter); 2. *P. brevicaulis* ($7\text{--}10\ \mu$ by $5.7\text{--}6.8\ \mu$); 3. *P. purpurogenum* ($2.8\text{--}3.3\ \mu$ by $2\ \mu$); 4. *P. claviforme* ($3\ \mu$ by $2\ \mu$); 5. *P. rubrum* ($2.8\text{--}3.5\ \mu$ in diameter); 6. *P. italicum* ($4\text{--}5\ \mu$ by $2\text{--}3\ \mu$); 7. *P. olivaceum* ($6\text{--}10\ \mu$ by $4\text{--}6\ \mu$); 8. *P. luteum* ($2.3\text{--}3\ \mu$ by $1.4\text{--}2\ \mu$); 9. *P. glaucum* ($2.5\text{--}3\ \mu$ in diameter).

Measured on growths from pure cultures on wort gelatin.

shaped. The mycelium is septate and usually blue-green in color. Toward the tip of the threads branching occurs to such an extent that a thick tuft is formed. At the tip of each of them are formed the spores or conidia. These conidia are formed in great numbers and finally break off to be carried by air and water currents and on other objects. Along with the color, the general shape of this mass of conidia-

bearing hyphae, called coremia, is used in classification of these fungi. The hyphae are septate.

Penicillium roqueforti.—This species is used for the preparation of roquefort cheese, as its name indicates. This type of cheese is filled with bluish-green masses which, if examined microscopically, appear to contain a great number of spores. Unlike camembert cheese, the preparation of which is described in another paragraph, *Penicillium roqueforti* grows within the cheese and ripens the mass in this manner. Roquefort cheese is made in somewhat the following manner. The mold to be used as the starter is cultivated on some nutrient material



FIG. 54.—*Penicillium brevicaulis* Saccardo. (After Thom.)

a, conidiophores and simple conidial chains with spores still smooth ($\times 900$); *b*, *f*, more complex conidial fructifications ($\times 900$); *c*, two young conidial chains, showing thick walls of spores ($\times 1,400$); *d*, *e*, conidia after becoming echinulate ($\times 1,400$); *g*, *h*, *j*, sketches of forms and habit of conidial fructifications ($\times 140$); *g*, from an old culture, sessile or almost so; *h* and *j* show trailing hyphae and a rope of hyphae with lateral conidiophores; *k*, germinated conidium where the old spore wall lies empty beside a growing cell ($\times 1,400$).

such as bread, in order to secure a good crop of spores with which to seed the curd from which the cheese is made. This curd is prepared from milk with rennin. The whey is taken off, leaving the solid portions of the milk. The curd is then spread out in a thin layer over which is sprinkled a little of the mold starter. Alternate layers of curd and mold starter are used until the cheese maker has a cake of the desired size. It is then pressed into shape and placed in a machine which punches it full of holes. These holes are necessary to allow the ingress of air essential for the growth of the mold. In this manner the mold is propagated within the cheese and, in its metabolic activities, gives rise to

those compounds characteristic of roquefort cheese. The home of roquefort cheese is in southern France. This mold was characterized by Thom as follows:

Colonies on potato agar or lactose gelatin quickly turning green, becoming a dirty brown when old, velvety strict, indeterminately spreading by large main radiating branching hyphae, giving a somewhat uneven or indefinite margin, which gets a white, fibrous, almost spider-web appearance from its alternation of submerged parts of hyphae with short prostrate aerial loops. Reverse of colony yellowish-white. Conidiophores arising separately and in acropetal succession from the growing parts of submerged hyphae (comparatively few aerial parts, but some), 200–300 μ septate.

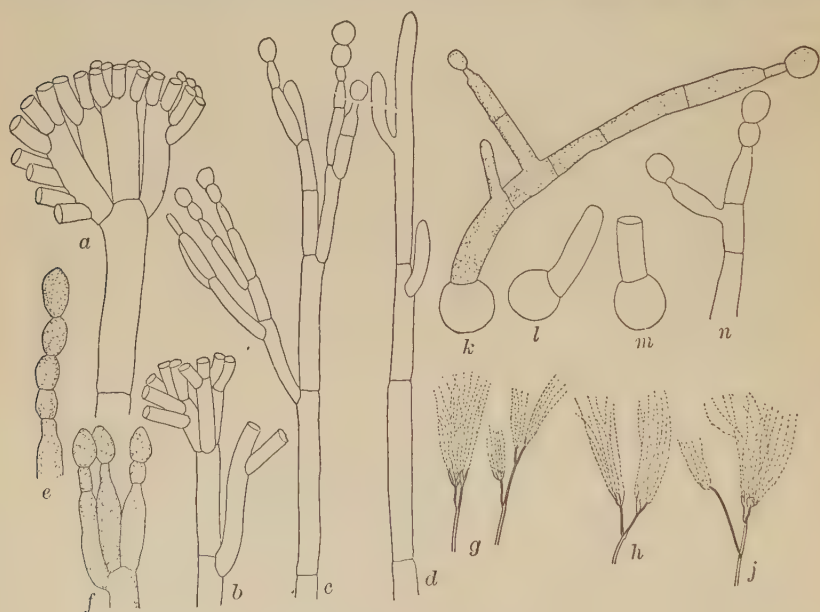


FIG. 55.—Roquefort Penicillium. (*P. roqueforti*). (After Thom.)

a, Part of conidiophore and of bas of fructification, highly magnified, showing the production of basidia on the sides as well as at the apex of the basidiophore; *b*, *c*, other types of branching; *d*, young conidiophore just branching; *e*, *f*, basidia and the formation of conidia, highly magnified; *g*, *h*, *j*, diagrams of types of fructification as seen under low power ($\times 80$); *k*, *l*, *m*, *n*, germination of conidia and new conidia produced directly on the first hyphae.

Fructification 90–120 μ or at times 160 by 30–60 μ at broadest place, usually appearing double by the divergence of the lowest branch; branchlets (“basidiophores”) irregularly verticillate, bearing crowded verticils of appressed conidiiferous cells (basidia), 9–11 by 2.5 μ with long, divergent chains of conidia. Conidia bluish-green, cylindrical to globose, smooth, rather firm-walled, 4–5 μ in diameter, germinating by a straight tube. Colonies do not liquefy sugar-gelatin, though they soften it somewhat. Fungus on plain gelatin or potato agar changes litmus from red to blue very rapidly and strongly almost from the beginning of the growth. Fruiting period short,

but one crop of spores upon the mycelium. Cosmopolitan and omnivorous, or nearly so. Characteristic of Roquefort and related types of cheese.

Penicillium camemberti.—This species was characterized as follows by Thom (1910):

Colonies on potato agar or lactose gelatin effused; white (sometimes yellowish white), changing in five to eight days to gray-green (glaucous); surface of colony floccose, of loosely felted hyphae about 5μ in diameter, reverse of colony yellowish white; conidiophores $300\text{--}800\mu$ in length, $3\text{--}4\mu$ in diameter, septate, cells thin-walled, often collapsing in age, arising as branches of aerial hyphae; fructification sometimes 175μ in length, but usually much less, consisting commonly of one main branch and one lateral branch, sparingly branched to produce rather few conidiiferous cells which bear long loosely divergent chains of conidia. Conidiiferous cells $8\text{--}11$ by $2.4\text{--}3\mu$. Conidia at first cylindrical, then elliptical, and finally globose when ripe, smooth bluish-green by transmitted light, thin-walled and commonly guttulate, $4.5\text{--}5.5\mu$ in diameter, swelling in germination to $8\text{--}10\mu$. Germ tubes one to several. Cells of mycelium about 5 by $20\text{--}40\mu$. Liquefies lactose gelatin only under center of colony. Produces a strong alkaline reaction in gelatin, free from sugar, but in sugar media produces a more or less persistent acid reaction. Growing and fruiting period, about two weeks. Fruits only upon exposed surfaces of the substrate; never produces spores in cavities not broadly open.

“Habitat, Camembert and other soft cheeses.”

KEY TO PENICILLIUM MOLDS. (AFTER THOM, 1910)

- A. Species fruiting typically by coremia (vertical and definite)
 - a. Coremia long ($3\text{--}15$ mm.)
 1. Conidial masses strictly terminal, olive green, fragrant . . . *P. claviforme*
 2. Upper third of coremia fertile, conidia green *P. duclauxii*.
 - aa. Coremia small
 1. Coremia definite, densely crowded, colony orange below
P. granulatum
 2. Coremiform characters indicated in cultures by clustering of conidiophores, definite coremia only in old cultures becoming large and definite on apples *P. expansum*
- AA. Species not or rarely producing coremia in culture:
 - B. Species constantly producing sclerotia or ascoginous masses
 - b. Producing asciginous masses, yellow or reddish *P. luteum*
 - bb. Sclerotia appearing as white masses in old cultures *P. italicum*
 - bbb. Sclerotia reddish or pink, globose or elliptical, 500 microns or less in less in diameter
 - c. Conidial fructification in column
 1. Column dense, long, sclerotia partially buried in substratum
P. No. 03
 2. Column formed of loose chains, sclerotia numerous, exposed
P. No. 29
 - cc. Conidial fructification of divergent chains
 1. Rapid liquefier, spores globose, $2\text{--}5$ microns *P. No. 31*
 2. Slow liquefier, spores elliptical, $3.5\text{--}4$ by $2.5\text{--}3$ microns
P. No. 32

BB. Sclerotia not (or rarely) produced under special conditions

C. Rapid liquefiers (abundant liquid in five to twelve days)

D. With definite strong ammoniacal odor:

1. Yellowish-brown avellaneous spores rough.....*P. brevicaulis*
2. White or cream, spores rough.....*P. brevicaulis*
3. White or cream, spores smooth.....*P. brevicaulis*, var. *glabr.*

DD. Without ammoniacal odor:

E. With yellow coloration of liquefied gelatin (*not of mycelium in reverse*)

1. Colonies small, conidiophores 100–150 microns in length

P. citrinum

2. Colonies broadly spreading conidiophores 250–300 microns

E. chrysogenum

EE. Without yellow color in liquefied medium (or slight traces only):

e. Colonies white, pink or salmon.....*P. roseum*

ee. Colonies some shade of green.

f. Colonies floccose, margin spreading by stolons.....*P. stoloniferum*

ff. Colonies velvety surface growth of fruiting hyphae only.

g. Conidiophores very short (100–200 microns)

1. *P. No. 12*2. *P. No. 37*

gg. Conidiophores longer (200–400 microns):

1. Conidiophores variously branched, reverse always colorless

*P. No. 24*2. Conidiophores each with a verticil of branches—each branch bearing a columnar fructification—reverse and medium darkening in sugar media.....*P. atramentosum*

CC. Liquefaction of gelatin none or slower than 10–12 days, or only partial

h. Colonies yellowish-brown, spores elliptical.....*P. divaricatum*hh. Colonies white to lilac, slow liquefier, 14–16 days.....*P. lilacinum*

hhh. Colonies floccose or creamy white

1. Conidiophores long, typical penicillate branching

P. camemberti var. *rogeri*

2. Conidial chains borne upon short branches of floccose hyphae

P. No. 33

GG. Colonies some shade of green

H. Surface with hyphae definitely in ropes or trailing, bearing numerous conidiophores, as short branches distinctly traceable to their origin in such hyphae

i. Colonies usually red below and reddening the substratum

1. Fruiting areas dark green.....*P. funiculosum*2. Fruiting areas mixed yellow and green.....*P. pinophilum*

ii. Colonies not producing red color

1. Colonies gray rarely greenish, very loose floccose....*P. intricatum*

2. Colonies green, conidial chains in simple compact column

*P. No. 48*3. Colonies gray to green, hyphae scattered, creeping...*P. decumbens*

HH. Surface hyphae, not in well-defined ropes, non trailing

j. Surface hyphae woven floccose, course of hyphae not traceable

1. Gray-green, long conidiophores, no odor.....*P. camamberti*2. Gray green, shorter conidiophores, strong odor.....*P. biforme*

jj. Surface growth at margin, simple conidiophores, in older parts both hyphae and conidiophores

1. Gray-greenish, branching of conidiophores rather loose, odor none or slight.....*P. No. 22*

2. Green, conidial fructifications rather compact, odor definite "moldy".....*P. commune*

[NOTE. There is probably a number of races in this group.]

jjj. Fruiting surface velvety—of simple conidiophores or conidiophores borne so close to surface of substratum as to appear simple

k. Conidial mass a dense column of conidial chains

1. Column from a single verticil of basidia.....*P. spinulosum*

2. Column from a verticil of branchlets with verticillate cells and chains.....*P. rubrum*

kk. Elements of conidial fructification not in column

1. Conidia smooth:

1. Green, broadly speaking, ripe conidia globose, 4-5 microns.....*P. roqueforti*

2. Green, less spreading, conidia elliptical, medium commonly purpled.....*P. purpurogenum*

3. Gray or olive green, conidia.....5-7 by 3-5 microns
P. digitatum

ll. Conidial delicate rugulose.....*P. rugulosum*

SPECIES OF PENICILLIA DETERMINABLE FROM THE SUBSTRATA (After Thom, 1910)

[In these species the substratum establishes a presumption of identity.]

Cheese (Camembert and Brie)

1. Floccose, white, unchangeable, no odor.....*P. camemberti* var. *rogeri*

2. Floccose, white to gray-green, no odor.....*P. camemberti*

3. Powdery, yellowish-white, spores smooth, ammoniacal odor

P. brevicaulis var. *Glabr.*

4. Powdery, yellowish-white, spores, tuberculate, ammoniacal odor

P. brevicaulis var. *album*

5. Forming yellowish-brown areas, spores rough, ammoniacal odor...*P. brevicaulis*

Cheese, roquefort

1. Green streaks inside the cheese.....*P. roqueforti*

Citrus fruits:

1. Colonies of mold blue-green.....*P. italicum*

2. Colonies of mold olive-green.....*P. digitatum-olivaceum*

Pomaceous fruits:

1. Blue-green colonies finally producing coremia.....*P. expansum*

Polyporaceae (Boleti, Polypori, etc.)

1. Colonies green (yellowish-green) spreading by stolons.....*P. stoloniferum*

Wood (pine)

Producing orange to red stains in pine wood.....*P. pinophilum*

ASPERGILLUS MOLDS

The mycelium of the *Aspergilli* is usually septate. The fertile hyphae are slender but thicken toward the tips to give a club-shaped appearance. About this club-shaped tip, called a basidium, are formed small projec-

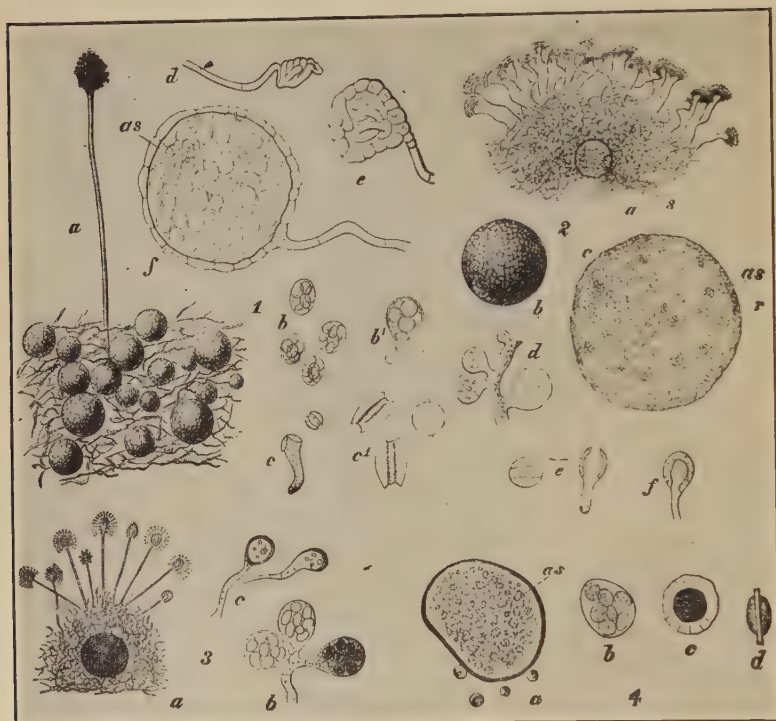


FIG. 56.—Ascospores of Four Species of *Aspergillus*. (After Wehmer in Lafar's *Handbuch der Technischen Mykologie*. By Permission of Gustav Fischer.)

Division 1.—Perithecia of *A. glaucus* resting freely on the substratum (a), also development of the perithecium from the *Eurotium* coil (d, e); at f a ripe perithecium with young asci; b, isolated asci; c', ripe spores; c, the same germinating. (c, b', d, e, f, after de Bary; a, b, c', after Wehmer.)

Division 2.—Sclerotia, with shell, of *A. nidulans* (a) prepared in the detached state (b); and section, showing sheath and asci (c); d, young asci; e, spores, one with germinating tube; f, swollen hypha of shell, with greatly thickened wall. (After Eidam).

Division 3.—Perithecia with shell, *A. Rehmii*, with separately prepared asci (b), and shell hyphae (c). (After Zukal.)

Division 4.—A Perithecium detached from the mycelial envelope of *A. fumigatus*, in section (a) containing asci (as); b, isolated ascus; c and d, spores with epidermal ridge, viewed from above (c) and from the side (d). (After Grijns.)

tions called sterigmata, at the tips of which are formed chains of conidia. The whole surface of the basidium is thickly covered with sterigmata and their chains of spores or conidia.

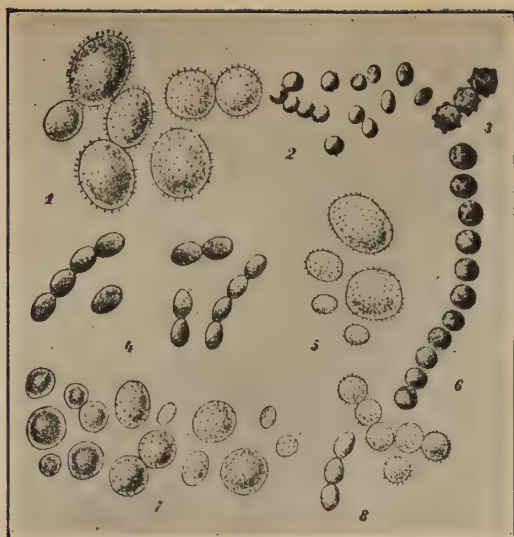


FIG. 57.—Conidia of *Aspergillus*. (After Wehmer in Lafar's *Handbuch der Technischen Mykologie*. By Permission of Gustav Fischer.)

All of approximately equal magnification, showing the differences in form and dimensions. *A. glaucus* (1). *A. fumigatus* (2), *A. niger* (3), *A. clavatus* (4), *A. Tokelau* (5), *A. varians* (6), *A. Oryzae* (7), *A. Wentii* (8).



FIG. 58.—*Aspergillus glaucus*. (From Lafar's *Handbuch der Technischen Mykologie*. By Permission of Gustav Fischer.)

Conidiophores (1, 2), sterigmata (3) and conidia (4). A portion of mycelium, with overlying perithecia and a conidiophore (magnified) is shown at 5, whilst 6 gives the natural size. Sections of perithecia are given at 7, with young asci (as) and first stages of development (*Eurotium coil*); isolated asci (8) and detached spores (9), germinating at c. (1, 7, 8, 9 (in part) after de Bary, the rest after Wehmer.)

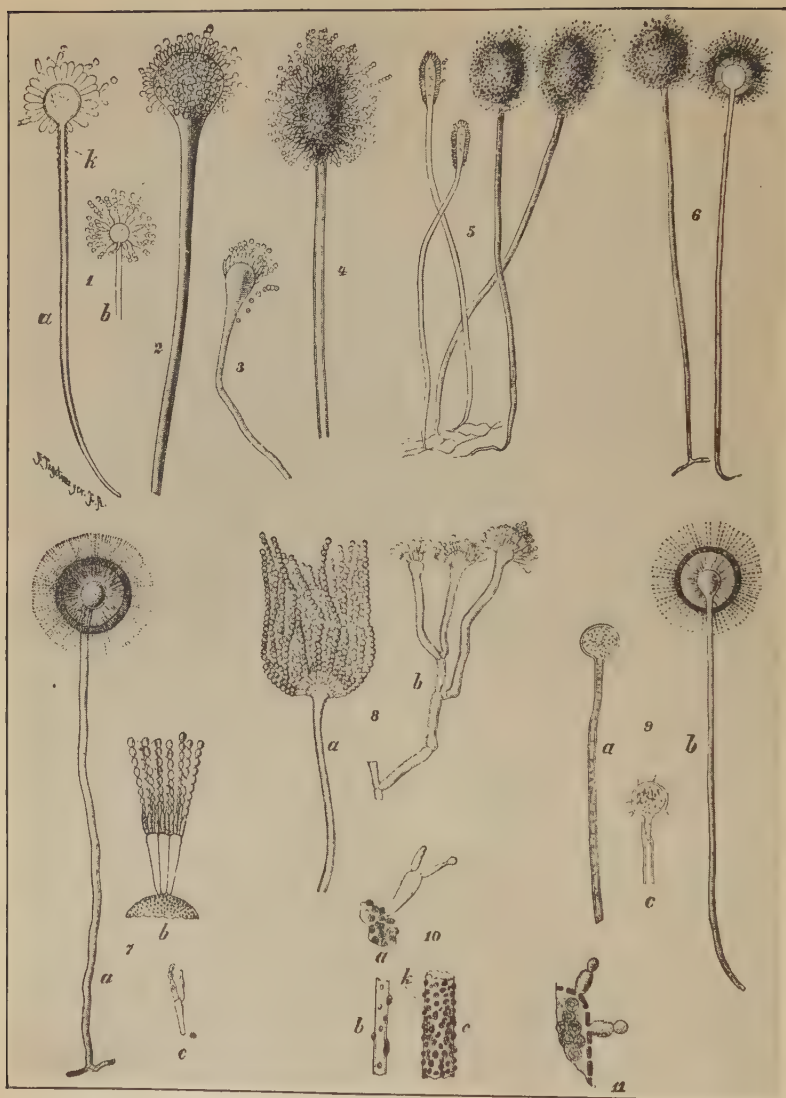


FIG. 59.—Conidiophores of *Aspergillus*. (After Wehmer in Lafar's *Handbuch der technischen Mykologie*. By permission of Gustav Fischer.)

Heads, globules and sterigmata of *A. Ostianus* (1), *A. glaucus* (2), *A. fumigatus* (3), *A. varians* (4), *A. clavatus* (5), *A. Wentii* (6), *A. sulfureus* (7), *A. nidulans* (8), and *A. candidus* (9). Excretion of granules from stalk and globule in *A. Ostianus* (10). Old sterigmata and globule of *A. candidus* (9, a-c). Fragment of globule from *A. giganteus* (high and medium adjustment combined). (7 after Zopf, 8 after Eidam, the rest after Wehmer.)

Aspergillus glaucus.—This is one of the commonest species. Its color is gray-green.

Aspergillus glaucus.—Conidial tufts when young bright green to verdigris green, later becoming darker, and finally becoming gray-green to gray-brown; hyphae hyaline, often in age yellow to brown, about 3μ thick; conidiophores erect, unbranched, 1–2 mm. high, 14μ or more thick, hyaline, beset with radiating, crowded, simple, short sterigmata that are 10– 14μ long by $5\text{--}7\mu$ thick; conidia in chains, globose or somewhat ovate, smooth, later mostly fine granular, mostly $7\text{--}10\mu$ in diameter, maximum 15μ .

Aspergillus globosus.—Conidiophores singly or aggregated in tufts; tufts olive-green, attaining a maximum height of 0.7 mm., and 0.4 mm. in diameter; conidiophores erect, septate, unbranched, $250\text{--}350\mu$ high by $4.5\text{--}5.5\mu$ thick, terminating in a subglobose to broad obovate swelling; swelling closely beset with branched sterig-

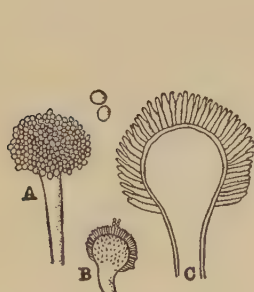


FIG. 60.—*Aspergillus fumigatus*, Fresenius. (After Jensen.)

A, Conidiophore and Conidial Head; B, Globose Swelling of Conidiophore Showing Sterigmata; C, Optical Section of Globose Swelling.

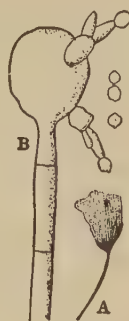


FIG. 61.—*Aspergillus globosus*. (After Jensen.)

A, Conidiophore and Conidial Fructification; B, Globose Swelling of a Conidiophore Showing Primary and Secondary Sterigmata and Spores.

mata, 12–30 by $10\text{--}15\mu$; primary sterigmata subglobose to broadly obovate, 5–6 by $4.5\text{--}5\mu$, very easily separating from swelling when placed in mounts; secondary sterigmata $5\text{--}8\mu$ long by $1.4\text{--}1.6\mu$ broad; conidial head long obovate to cylindrical, $120\text{--}225$ by $100\text{--}190\mu$; conidia globose, warty, tinged with green, $2\text{--}3\mu$ in diameter, in very long chains.

Aspergillus fumigatus.—Colonies orbicular, at first light green, finally becoming very dark green; hyphae creeping, branched, septate, hyaline, $1.5\text{--}6\mu$ thick; conidiophores $200\text{--}400\mu$ high, $3.5\text{--}6\mu$ thick, ascending, erect, hyaline, usually unbranched, uni-septate, toward the summit enlarging into a globose swelling of $10\text{--}20\mu$ in diameter; sterigmata cylindrical to clavate, hyaline, 6–8 by $1.5\text{--}2\mu$ simple, continuous; conidia broad ovate to perfectly globose, $2.5\text{--}2.5\mu$ in diameter, smooth, catenulate, chains short.

Aspergillus oryzae.—This species was isolated by Wehmer from the Japanese saké breweries. This species is active and a rapid destroyer of organic matter.

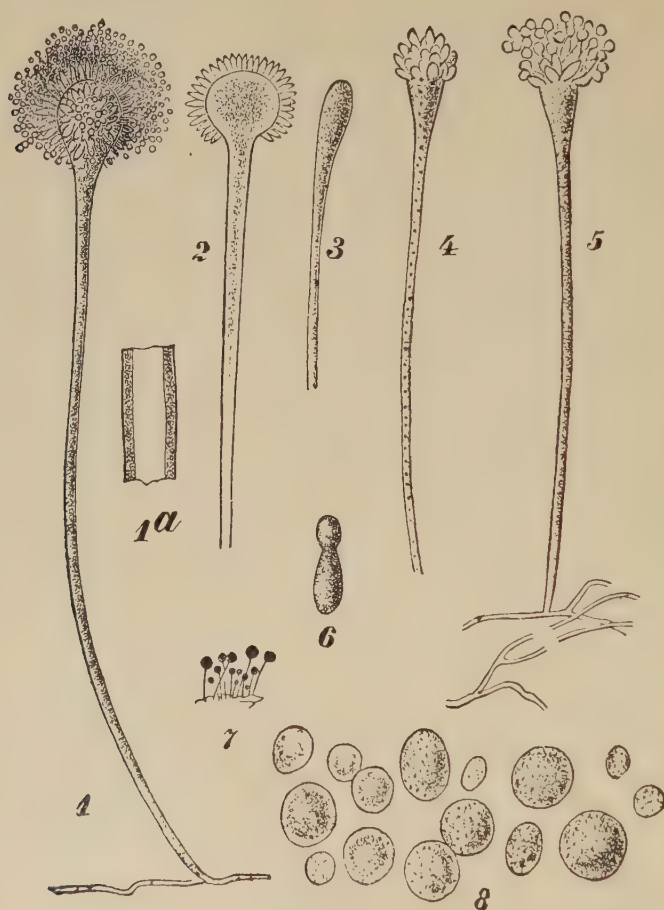


FIG. 62.—*Aspergillus oryzae*. (After Wehmer.)

1-2. Conidiophores with clavate and almost spherical globule. 2. In optical section. 3-5. Development of a small conidiophore, distention of the hypha, protrusion of sterigmata and incipient formation of conidia. 1a. Optical section of tough stem. 6. Sterigma. 7. Conidial herbage, slightly magnified. 8. Conidia. Approximate magn. of 1-5, 75; of 6, 400; of 8, 900.

BOTRYTIS

The conidiophores of *Botrytis* stand erect from a dark-colored mycelium. At the tip is a tuft-like formation of conidia. The group is perhaps sufficiently well characterized by descriptions of the following species by Jensen.

Botrytis fulva Link.—Colonies in form of a stratum, rather thick, spreading, yellow-brown, consisting of thickly intertwined or cobwebby hyphae; conidiophores much branched; conidia globose, 4–5.5 μ in diameter, with fine spines, yellow-brown.

Botrytis dichotoma Corda.—Colonies small, white with cinnamon-colored spots; conidiophores erect, closely forked, extreme branches blunt and slightly curved inward, hyaline, 12–14 μ thick, conidia produced in great numbers in the form of a spiral, globose, often angled from lateral pressure, yellow-cinnamon-brown.

Botrytis geophila Bonord.—Colonies gray, delicate; conidiophores short, septate, almost hyaline, upward dichotomously branched, with short blunt, forked end branches that appear as crutches; conidia small, globose, blackish.

Botrytis terrestris Jensen.—Colonies at first white, later gray; sterile hyphae creeping, hyaline, branched, septate, 1.5–3 μ in diameter; conidiophores erect, ascending, septate, branched, 2–3.5 μ in diameter, 50–200 μ high; primary and secondary branches verticillate, dichotomous, or alternate; conidia produced on the ends of the branches, forming more or less compact triangular clusters that average 20–25 μ , obovate, somewhat angled, uniform 2.5–3 by 3–4 μ , hyaline to light gray. Clusters of conidia separate very easily. Isolated from potato soil on experimental plants.

Botrytis cinerea.—Colonies diffuse, gray, gray-green, dark olive-green to brown-black, seldom brown or reddish-green, dusted gray from the conidia, loose or dense, up to 2 mm. high; conidiophores erect, unbranched or seldom branched, septate, 11–23 μ thick, with blackish-brown membrane, toward the summit almost hyaline, at the summit with several (3 or more) somewhat half-globose outgrowths (Auswüchsen) from which the conidia are formed singly on very fine wart-like projections. The tip of the conidiophore grows between the outgrowths (Warzen), whereby they become separated and placed laterally; occasionally in this position they still carry single conidia. The conidia stand so thickly on the projections (Auswüchsen) that thick heads are produced which soon fall off, ovate or elliptical to almost globose, on the base finely apiculate, 9–12 (up to 15 μ) long and 6.5–10 μ broad, with almost hyaline, hardly brownish membrane.

Botrytis epigaea.—Colonies far-spreading, floccose, at first white, then yellowish-brown to red-brown; hyphae creeping, septate, 11–16 μ thick, with brownish, thin, granulated membrane. From the principal hyphae are produced side branches that average 30–40 μ long; side branches are mostly without septa, with smooth, bright membrane, on the summit irregularly and slightly swollen and ending in a great number of wholly irregular, thin, 5–7 μ long, hyaline projections (Zähnen). The principal stem rarely ends similar to the side branches; conidia borne singly on the projections, globose, 3.5–5.5 μ in diameter, hyaline, uniguttulate.

HYPHOMYCETES

MUCEDINACEAE

Saksia albicans.—Colony orbicular, dense, snow-white, raised, margin irregular, deeply crenate; mycelium hyaline, submerged and aerial; submerged mycelium consisting of yeast-like cells usually swollen at both ends, constricted in the middle, occasionally pyriform, guttulate throughout the cell 7–15 by 2–8 μ . Aerial hyphae 26–86 μ long by 2–4 μ thick, often irregular in diameter, gradually attenuate toward tip, guttulate, with yeast-like cells toward base.

The original states that the mycelium gives off round, oval, or pyriform spores, similar to *Mycoderma* but distinguished from this genus in that short mycelial threads arise from the cells or spores. Size of these spores is 0.6–4 by 0.5 μ . The

author further states that he had a culture which produced no spores and no such aggregations as described. Our culture so far has not been observed to produce the spores. The author gives dimensions of yeast-like cells as $10\text{--}20\mu$ long by $2\text{--}10\mu$ in diameter, and aerial hyphæ $50\text{--}200\mu$ long by $2\text{--}10\mu$ wide. Our form, as already observed, does not quite agree with these measurements.

OIDIUM

This mold is set apart from some of its neighbors by the lack of well-defined fruiting bodies. It is a septate mold. Each of the threads is

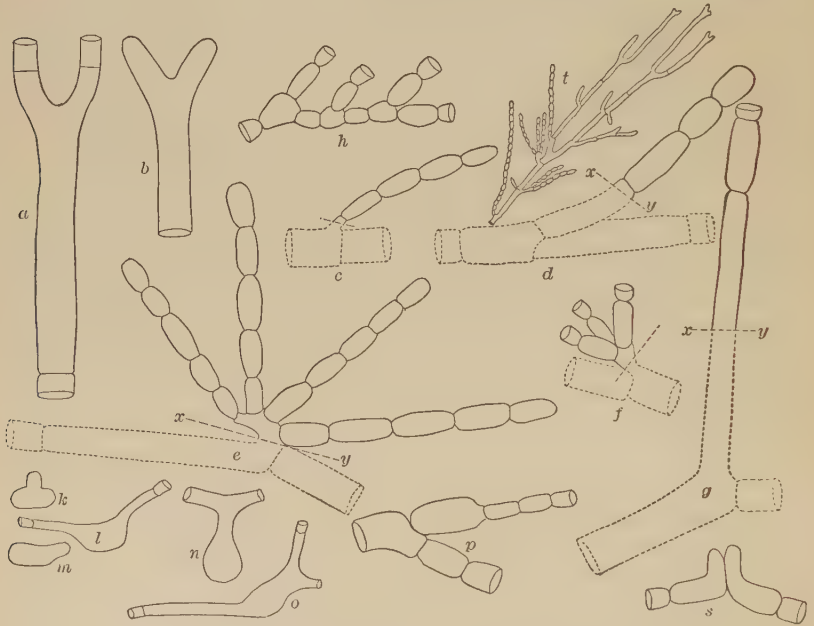


FIG. 63.—*Oidium lactis*. (After Thom.)

a, b. Dichotomous branching of growing hyphæ; *c, d, g*, simple chains of oidia breaking through substratum at dotted line *x-y*, dotted portions submerged; *e, f*, chains of oidia from a branching outgrowth of a submerged cell; *h*, branching chain of oidia; *k, l, m, n, o, p, s*, types of germination of oidia under varying conditions; *t*, diagram of a portion of a colony showing habit of *Oidium lactis* as seen in culture media.

able to break up into "oidia," a unit quite comparable to the spore or conidium. The oidia are usually rectangular with rounded corners.

Oidium lactis.—This fungus is quite widely distributed in nature. It is easily secured from sour milk on which it grows as a velvety layer. Jensen described the species as follows:

Colonies far-spreading, membranaceous, velvety, pure white, often becoming a thick covering over substratum; hyphæ simple or branched,

creeping or somewhat ascending, hyaline, of variable length and breadth, mostly $6\text{--}12\mu$ broad, breaking up into irregular pieces that are to be considered as spores; spores cylindrical to ovate, often also globose, or somewhat irregular in form, mostly $6\text{--}20\mu$ long.

ALTERNARIA

Alternaria are characterized especially by a muriform conidium.

Alternaria fasciculata.—Conidiophores brown, erect or ascending, irregularly curved, solitary or caespitose, septate, diameter uniform, $40\text{--}130\mu$ long by 3μ wide; conidia dark brown, oblong ovate, minutely apiculate, $9\text{--}14\mu$ wide by $35\text{--}90\mu$ long, endochrome transversely 2–7 septate with usually several longitudinal septa, the apical cell short or elongated into a straight, somewhat hyaline beak.

Alternaria humicola.—Colonies at maturity orbicular, black-green; fertile hyphae well developed, hyaline, articulate, $3\text{--}5\mu$ in diameter, racemosely branched; conidia polymorphic, cylindrical, obclavate, oblong, lageniform, at first hyaline, later honey-colored, thin, dark, finally black-green and fuliginous, variable in size, maximum 16 by 50μ , 3–7 septate, muriform, in advanced age dense and very finely roughened, slightly or non-constricted at septa.

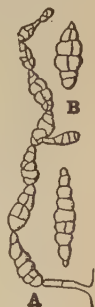


FIG. 64.—*Alternaria fasciculata*. (After Jensen, 1912)

A, Conidiophore and Conidial Chain; B, Conidia Enlarged.

FUSARIUM

Species of *Fusarium* are frequently encountered by microbiologists. They have been found to be important in the dry rot of potatoes and other vegetables. *Fusaria* form *macroconidia* although in certain species *microconidia* appear. These fungi usually grow well on ordinary media yielding a vigorous dense growth. The spores germinate much as do other spores.

ACTINOMYCES

These organisms are characterized in the last two classifications which have been presented in Chapter VI. They are mold-like organisms which include both parasitic and saprophytic species.

MONILIA

Monilia species are much like the yeasts in many respects. They tend to form long cells which appear as threads under the microscope. The group is not well characterized. Interest in the past was drawn to the group by a very interesting member *Monilia candida* which was identified by Hansen. He found the fungus rather widely distributed on fruits, soil and cow dung. The fungus grows well on wort showing an abundant film formation and vigorous alcoholic fermentation at the

same time. Another interesting fact about this fungus is that it ferments cane sugar; yet several eminent biologists have not been able to

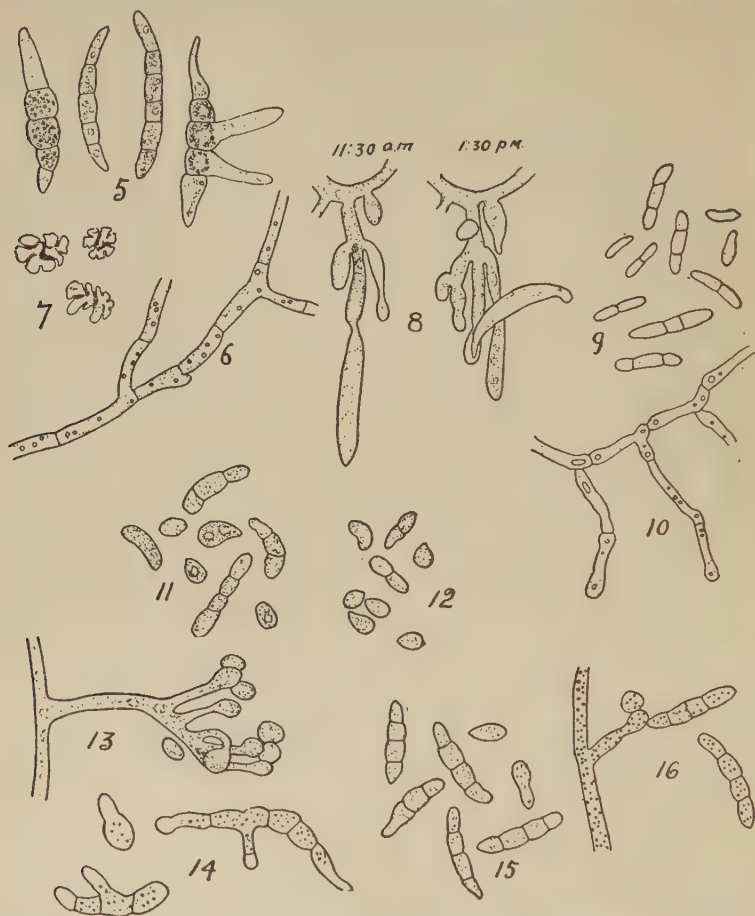


FIG. 65.—Showing Various Stages in the Development of *Fusarium*. (After Burrill and Barrett.)

FIG. 5.—Macroconidia of *Fusarium II*, some of which are beginning to germinate. FIG. 6.—Mycelium of the same fungus. FIG. 7.—Starch grains from a corn kernel infected with *Fusarium II*, showing the corrosive effect. FIG. 8.—Sporophores of *Fusarium II*, drawn at 11:30 a.m. and at 1:30 p.m. to show the rate of development of the spores in culture. FIG. 9.—Microconidia and macroconidia of *Fusarium III*, from culture. FIG. 10.—Mycelium of the same from a corn kernel. FIG. 11.—Microconidia and macroconidia of *Fusarium I* from a prune agar plate. FIG. 12.—Same from an infected ear of corn. FIG. 13.—A spore-producing hypha from a young prune juice culture. FIG. 14.—Germinating spores of *Fusarium I*, from a dried, diseased embryo ear of corn. FIG. 16.—A hyphal branch of *Fusarium I*, producing both microconidia and macroconidia.

demonstrate invertase which was excreted by the cell. Some evidence is available to show that an intracellular invertase is formed and probably retained within the cell.

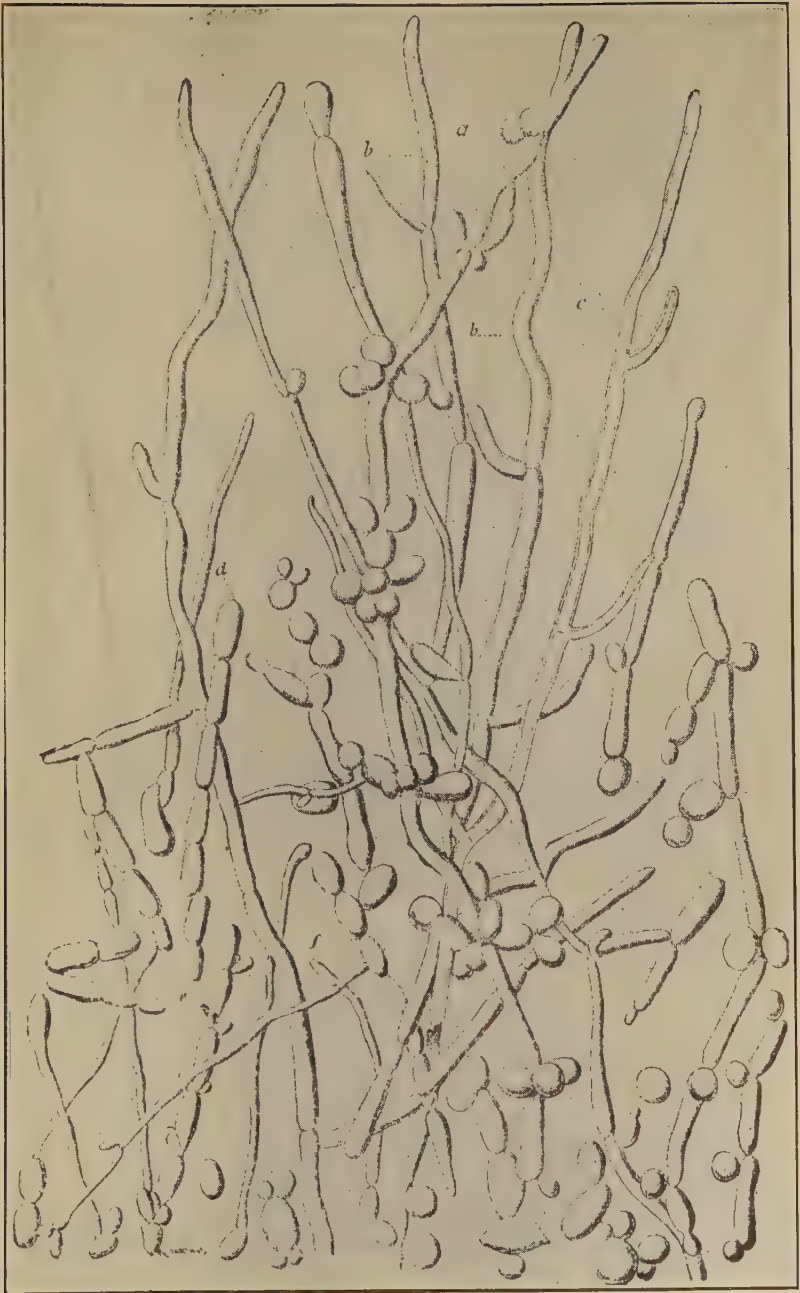


FIG. 65.—*Monilia candida*. Showing the Mold-like Cells from Certain Cultures.
(After Hansen.)

PATHOGENIC MOLDS AND MOLD-LIKE FUNGI

Among the molds, as among the bacteria, certain species are pathogenic for man and other animals. The lesions caused by the molds are not unlike those caused by bacteria. The diseases caused by molds and other fungi are often called mycoses, the specific mycosis being designated either from the etiologic agent or the type of infections. Thus, such names as bronchiomycosis, actinomycosis, etc., are secured. A few of the more common infections caused by fungi will be discussed.



FIG. 67.—*Monilia candida*. (After Hansen.)

A. Cells from Cultures in Beer Wort; B, Cells from a Film on the Surface of Liquid Media.

Ringworm.—This is a very communicable skin infection and an insidious one to treat. It is caused by a fungus *Trichophyton tonsurans* which burrows into the skin, where different agents, usually employed in its treatment, cannot reach it. It is usually treated with fungicides such as turpentine ointment, X-rays, or ultraviolet light. The appearance of the lesion caused early investigators to name the infection ringworm. There is a marked tendency for the lesion to heal at the center and spread at the periphery. This gives the ring appearance. When the fungus infects the hairy portions of the body the infections are treated with difficulty. Other infections are caused by the same agent which causes ringworm. Barber's itch, herpes tonsurans, etc., are caused by *Trichophyton tonsurans* or related species. These fungi are also pathogenic for other animals.

The fungus causing ringworm is especially prone to attack the hands and feet. Surgeon-General Cumming of the United States Public Health Service has stated that probably at least one half of all adults suffer from it at some time. Those who use swimming pools, athletic clubs, etc., or any place where there is a common dressing room may have the infection usually on the feet. The parasite is quite resistant and may live and grow on other agents than the human body. Cumming stated that fifteen minutes boiling were required to kill it. Such agents as bath mats, stools and the like may spread the parasite. Infected individuals should not use bath mats but paper which may be burned. It is obvious that strict cleanliness is necessary. Dressing-rooms should be thoroughly cleaned and disinfected.

Aspergilloses.—Several species of *Aspergillus* molds cause infection in man and other animals. One of the best-known species in this respect is *Aspergillus fumigatus*. The disease caused by this mold in fowls is called aspergillosis and is characterized by the formation of nodules on the mucous lining of the upper respiratory tract. Each of these nodules contains a growth of mold about which is formed a layer of animal cells. As growth increases the surface of the mucosa becomes greenish in color. *Aspergillus fumigatus* which causes this infection is a resistant species, the spores of which seem to be quite widely spread in nature. The spores are probably disseminated in the food and drink of the fowls. It has been stated that foods which are exceptionally dusty and dirty are more liable to cause infection. After the fungus has become established it propagates itself to such an extent that the respiratory tract is filled, causing great difficulty in breathing and finally the bird suffocates. The characteristics of the fungus have been given on page 155.

Members of the genus *Aspergillus* also cause infections among human beings. They may cause lung infections which are much like tuberculosis. Skin infections have also been found to be due to *Aspergilli*.

Alternaria Infections.—*Alternaria* have been found to be present in infections in the respiratory tract especially those of chronic pulmonary type. Some of these infections have been inconclusively diagnosed but since no other etiologic agent could be found *Alternaria* has been regarded as the important causal agent.

Actinomycosis.—Infections with *Actinomyces* are known as actinomycosis, lumpy jaw, wooden tongue, etc. It is a fairly common infection in animals of certain countries. Human beings may also not infrequently become infected with the organism *Actinomyces bovis*. The disease is an insidious one to treat. It is usually characterized by the formation of much pus and a sloughing away of tissue and bones. The organisms causing actinomycosis are believed to be widespread in nature in grains and similar materials. They get into the subject through an abrasion or

into the respiratory tract by inhalation. The disease is not epidemic but appears as isolated cases. This is perhaps one reason why diagnosis is delayed and the treatment unsatisfactory. *Actinomyces necrophorus*, or an organism very much like it, has been isolated from cases of puerperal infection.

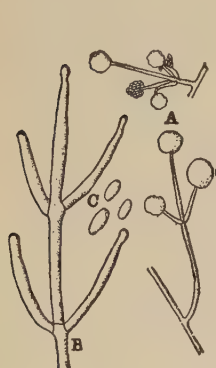


FIG. 68.—*Acrostalagmus albus* Preuss var. *varius*. (After Jensen.)

A, Conidiophores and Conidial Heads.

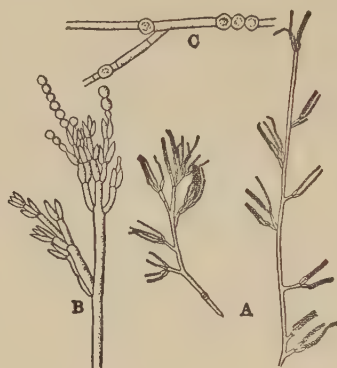


FIG. 69.—*Spicaria simplicissima* Oud. (After Jensen.)

A, Conidiophores and Conidial Fructification; B, Same Enlarged; C, Chlamydospores.



FIG. 76.—*Mycogone nigra*. (Morgan); (After Jensen.)

A, Chlamydospore; B, Conidiophores and Conidia; C, Germinating Conidia.



FIG. 71.—*Hormodendrum cladosporioides*. (Freas.) Sacc. (After Jensen.)

A, Conidiophores and Conidia; B, Ultimate Branches of Conidiophore and Conidia; C, Old Articulate Hypha.



FIG. 72.—*Botrytis terrestris*; Conidiophores and Conidial Clusters. (After Jensen.)

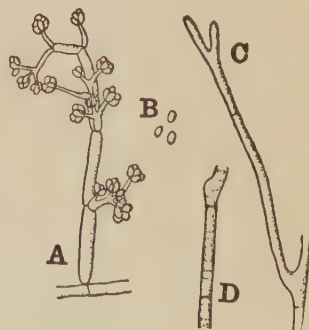


FIG. 73.—*Trichoderma Koningi* Oud. (After Jensen.)

A, Sporangioophore, Showing Method of Branching and Conidial Heads; B, spores; C, Showing Method of Branching of Young Hypha; D, Showing Old Vacuolate Hypha.

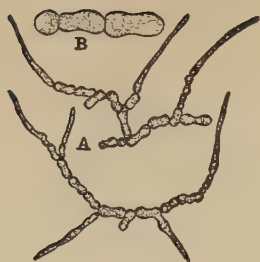


FIG. 74.—*Sachsia albicans*,
C. Bay. (After Jensen.)

A, Hyphæ, Showing Method
of Development and Branch-
ing; B, Segments of Hypha
Enlarged.



FIG. 76. — *Stachybotrys cylindrospora*: Conidiophore, Basidial Cells, and Conidia. (After Jensen.)

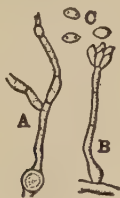


FIG. 77.—*Stachybotrys atra*
Cda. (After Jensen.)

A, Hypha with Chlamydospores; B, Conidiophores
and Basidial Cells; C, Conidia.



FIG. 75.—*Hormodendrum hordei* Bruhne. (After Jensen.)

A, Conidiophore
and Conidia; B,
Conidia Enlarged.



FIG. 78. — *Trichothecium roseum* Link: Conidiophore and Conidia. (After Jensen.)

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CHAPTER VIII

THE YEASTS AND RELATED ORGANISMS

The term yeast is used with different meanings. Generally two characteristics are emphasized, the formation of appreciable amounts of alcohol and carbon dioxide and reproduction by a process spoken of as budding. These are unsafe criteria, however, to use for grouping an organism with yeasts. Many organisms in quite different genera form alcohol and some reproduce by budding. It is impossible to establish the limits of any group without finding that border-line organisms cause confusion. For convenience, perhaps, the yeasts are roughly divided into two groups, true yeasts and pseudo- or false yeasts. The former produce ascospores while the latter do not. The pseudo-yeasts are also considered as fungi imperfecti.

Botanical Position of the Yeasts.—The yeasts are often spoken of as budding fungi. We have discussed the term *fungus* on earlier pages and the term budding is defined in a later paragraph. They are *Myromycetes* which have the ability of forming endospores or ascospores in a sac called an ascus. At first thought, one might regard this as the same phenomena which we have discussed among the molds, i.e., where a sporangium was formed to contain many conidia, or spores. However, they are different phenomena. In the sporangium, there are many nuclei concerned, while in the ascus, only one when the process of sporulation begins. On account of the presence of the ascus with its ascospores, the yeasts are *Ascomycetes*. The evolutionary development of the yeasts is an interesting subject but one with which we need not be concerned.

Shapes of Yeast Cells.—The yeasts are unicellular organisms varying greatly in shape. Most

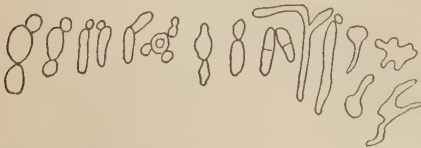


FIG. 79.—Showing the Different Shapes of Yeast Cells. (After Guilliermond.)

of them have cells which are round or oval. One species has sausage-shaped cells. The cell shape is one of the first differential characteristics which may be used for the separation of one species from another. A number of names of shapes have been accepted as

types and are used conveniently when one wishes to indicate shapes of yeast cells.

cerevisiae = round or globular, isolated cells.
 ellipsoideus = elliptical cells.
 pastorianus = sausage-shaped.
 apiculatus = lemon-shaped cells.

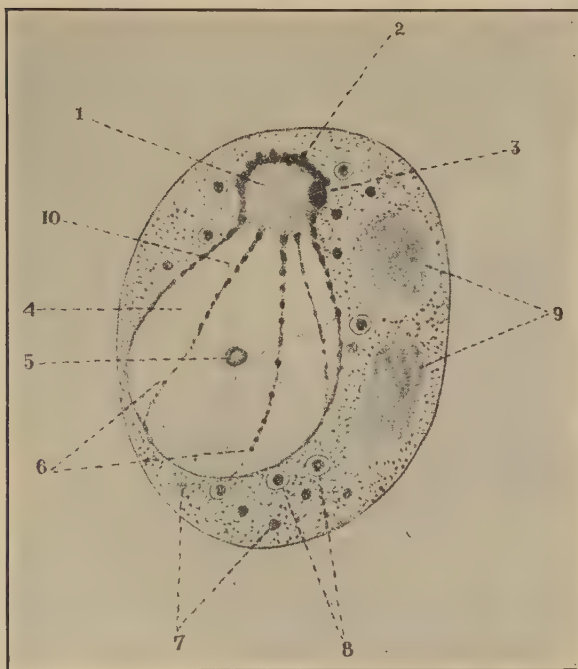


FIG. 80.—Diagram of Yeast Cell. (After Wager, 1911.)

1. Nucleolus. 2. Peripheral layer of chromatin. 3. Chromatin patch on one side of nucleolus. 4. Nuclear vacuole. 5. Central volutin granule in the vacuole. 6. Chromatin network. 7. Granules of fatty substance. 8. Volutin granules. 9. Glycogen vacuoles. 10. Delicate suspending threads for the central volutin granule.

Mycelial Structures.—Some yeasts show a marked tendency toward what are called mycelial formations. These are thread-like and give the yeast the appearance of molds. This tendency, as can be easily understood, caused confusion among the early botanists who were trying to classify and identify the various species. Mycelial formations appear especially in pellicles, veils or films on the top of fermenting liquids—when they are formed. *Mycoderma* species are typical film formers. They produce a film in a very short time. Some *Saccharomyces* form a typical film but it requires a longer time. Different explanations

have been offered for the formation of films by certain species. Some have suggested that the ability to grow on the surface was due to the formation of fat by means of which the cells resisted wetting. Others have believed that it might be due to a demand for free oxygen.

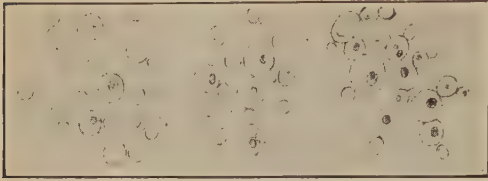


FIG. 81.—Showing Pastorianus-Shaped Yeast Cells. (After Wehmer.)

the organs usually found in cells. Yeasts are especially useful organisms for the study of cytology on account of their rapid growth and ease of visibility. The size of the cell allows quite accurate studies to be made with it. Many of the data secured with the yeast cell have been used as bases for deduction and experiment with the smaller bacterial cells.

Cell Membrane.—Yeast cells possess a thick easily demonstrated cell wall. Some investigators have reported the presence of a special type of cellulose. The membrane on some yeasts may consist of two layers.

Nucleus.—The nucleus of yeasts is large and may take up a considerable portion of the interior of the cell. The nucleus is usually globular, at least in young cells, but may vary in shape as the cell grows older. The nucleus plays an important role in the reproduction of the cell since it undergoes a division, one part remaining in the mother cell and the other going into the bud if the species reproduces by budding.

Structure of Yeast Cell.—The yeast cell is a typical cell possessing all of

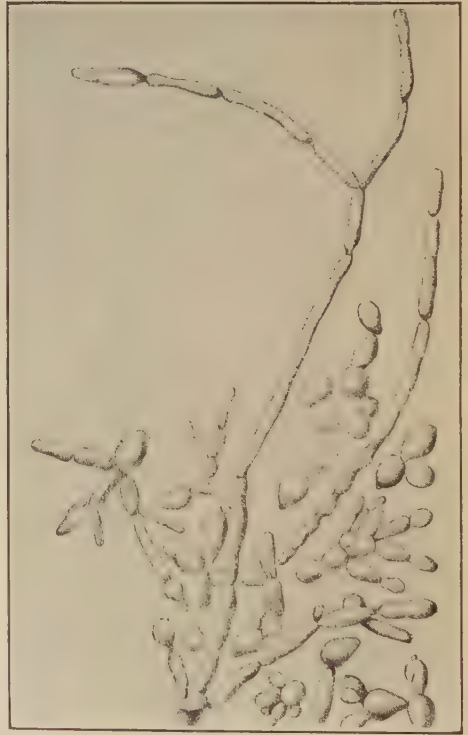


FIG. 82.—*Saccharomyces cerevisiae*. Cells from the Film of a Culture at 15°. (After Hansen.)

Vacuoles.—These organs are visible in yeast cells as slightly refractive bodies. When the vacuoles increase in number, or when they become quite large, it indicates exhaustion of the cell or lack of food. The vacuoles are filled with a liquid the exact nature of which is not clearly understood.

Granules.—Microscopic examination of a yeast cell usually reveals the presence of granules which appear as refractive units. They are distributed in the cytoplasm but may also appear in the vacuoles. They seem to be absent in young cells appearing in older cells especially when glycogen is reduced in amount.

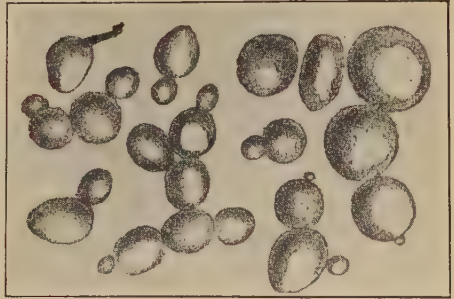


FIG. 83.—*Saccharomyces cerevisiae*. Showing the Cell Shapes of Cells from the Sediment of Young Cultures. (After Hansen.)

Ascospores and Ascosporulation.—The ascospores in the yeasts are synonymous to the endospores of bacteria. Among the yeasts ascospores are formed in a sac called an ascus. The yeasts, then, are *Ascomycetes*. Ascospores were first described by Schwann, but the conditions which influence their formation were determined by Hansen. The shape

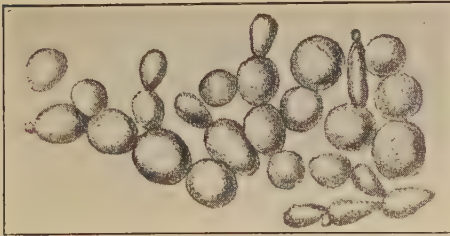


FIG. 84.—*Saccharomyces cerevisiae*. Showing the Cell Shapes in Films from Old Cultures. (After Hansen.)

of ascospores varies greatly among the different species. Their shape is used in the identification of species. Ascospores of members of the genus *Willia* have a derby-hat shape. Guilliermond has done much to emphasize the fact that the ascus may result from the fusion of two cells both of which function as gametes.

Two cells close to one another put out little projections which unite to form a copulation canal. The contents of one cell passes over into the other. The resulting cell in this case is a zygospore-like cell, the nucleus of which soon divides into the required number of parts to form the ascospores. It has also been shown that in certain species the ascospores within the ascus may also copulate. These fusions need not take up much of our time since the details may be determined in more advanced texts.

Hansen, to whom we are indebted for much of our information

on yeast cytology, reported the following conditions as influencing ascospore formation:

1. The cells should be healthy and well nourished.
2. There should be an abundant supply of air.
3. Water must be present.
4. Proper temperature must be maintained (about 25° C.).

Germination of Ascospores.—The ascospore is a resistant resting stage which remains in this state until favorable conditions exist when it may germinate and reproduce the vegetative stage in growth. Various methods are followed, depending on the species.

Properties of Ascospores.—The yeasts are somewhat less resistant to heat than the bacteria. The vegetative cells of yeasts are destroyed around 60° C., in a comparatively short time. The spores are a little more resistant. Most of them, however, are destroyed by temperatures of pasteurization (60–65° C.).

Reserve Materials.—Yeast cells, like the cells of other types of organisms, are able to put down appreciable amounts of reserve products, such as glycogen and fat. The former was discovered by the great French physiologist Claud Bernard as the carbohydrate in the liver of animals. Since it has somewhat the same function and interest that starch has in plants, it has often been called animal starch. Glycogen is regarded as a reserve material since in times of plenty the content increases, to decrease markedly when the cell is in a starved condition. Another reserve product in yeast cells is fat. The content varies greatly. Certain yeasts are known as fat yeasts since they are able to change simple compounds to fat. *Torula lipofera* has been described as such a species. A yeast-like fungus *Endomyces vernalis* is also a great fat producer. Guilliermond states that the fat content of old cells may reach as much as 20 per cent on the dry basis.

Reproduction of Yeasts.—Yeasts are unicellular microorganisms and reproduce with somewhat the same rapidity as the bacteria.

Budding.—This is a process by which certain yeasts reproduce. One cell is very small and appears on the side of the first cell as a little bud. The former is called the mother cell and the latter the daughter cell. The daughter cell increases in size until it has approached the size of the mother cell when it breaks off and continues growing until the adult stage for the species is attained. This daughter cell, now fully grown, may, in turn, form buds and thus function as a mother cell. Under proper conditions of food supply, temperature, etc., each mother cell will form many buds all about the cell. Quite often one may see the

daughter cells still attached to the mother cells, forming a clump of yeast cells.

Transverse Division (Partition).—This is quite another method of reproduction from the one just described. With budding the progeny are smaller and have to grow to the size of the cell which formed them. The method discussed here is called transverse division because each cell divides transversely to form two cells which are one-half the size of the original cell. The species which reproduce in this manner are called *Schizosaccharomycetes*. This division is accomplished by the appearance of the cross-wall in the middle of the cell after it has elongated. The appearance of the cross-wall is followed by a constriction at this point; the cell finally breaks giving the two cells. As was the case in budding, the cells often remained attached for a while giving clusters or clumps.

Habitat of Yeasts.—Like certain bacteria yeasts are widely distributed in nature. This question was studied by some of the early investi-

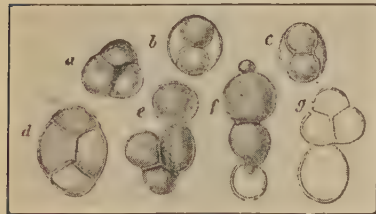


FIG. 86.—*Saccharomyces cerevisiae*. Showing the Early Stages in the Germination of Ascospores. (After Hansen.)

a, d, e, and g. Formation of Walls; e, f and g, The Walls Have Been Broken; An Ascospore with Several Chambers.

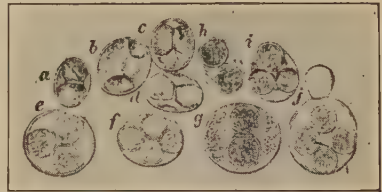


FIG. 85.—*Saccharomyces cerevisiae*. Showing the Early Stages in the Development of Ascospores. (After Hansen.)

a, b, c, d, and e. Rudiments of Ascospores with Indistinct Walls; f, g, h, i, and j, Mature Ascospores.

gators, one of whom, Brefeld, stated that they were especially common in excrement. The researches of Pasteur, however, gave a different aspect to the situation. Pasteur, after considerable study of the question, reported that yeasts were present on ripe grapes, which raised the question about where they spent the winter, when there were no grapes. It remained for E. C. Hansen, in 1880–1881, to answer the question. Hansen, working first with a species *Saccharomyces api-*

culatus found that it reached the soil by being washed from the fruit by rain, where it passed the winter. This fact was very satisfactorily proved by later investigations. The transportation to the skins of fruits is easily explained. They may be blown about in dust. One investigator found that insects played a rôle in helping

to disseminate the yeasts. They are probably as widely disseminated in nature as the bacteria.

Classification of Yeasts.—Yeasts are roughly divided into two great groups depending on whether ascospores are formed or not. True *Saccharomyces* form ascospores and reproduce by budding while *Schizosaccharomyces* form spores and reproduce by partition. Besides the above-mentioned group, there are the false or pseudo-yeasts which do not form ascospores. They may, however, reproduce by budding. Among the latter are the *Torulæ*, *Mycodermae*, etc., two genera which are not sharply differentiated.

The classification given below is Guilliermond's modification of Hansen's.

A. TRUE SACCHAROMYCETES¹

Family I. Saccharomycetaceae.

Reproduce usually by budding, sometimes by partition; each cell may develop into an ascus containing usually 1 to 4, rarely 12, ascospores.

Genus I. *Schizosaccharomyces* Lindner.

Yeasts multiplying by partition, asci often derived by copulation; 4 to 8 ascospores.

Genus II. *Zygosaccharomyces* Barker.

Asci preceded by an iso- or heterogamic copulation, ascospores with a thick membrane.

Genus III. *Debaromyces* Klöcker.

Asci derived from a copulation usually heterogamic; ascospores globular and provided with a verrucose membrane.

Genus IV. *Nadsonia* (*Guilliermondia*) Nadson.

Asci derived by budding from a cell which has been formed by heterogamic copulation.

Genus IX. *Saccharomyces*.

Ascospores germinate by budding, often forming a rudimentary mycelium.

Genus X. *Hansenia*.

Apiculate cells. Asci with single ascospore.

Genus XI. *Pichia*, Hansen.

Hemispherical ascospores.

Genus X. *Willia*.

Lemon-shaped ascospores or shaped like a derby hat.

¹ This classification is an abbreviated one taken from Guilliermond, "The Yeasts." Translated by F. W. Tanner. The complete classification may be consulted in that text.

B. PSEUDO-SACCHAROMYCETES

Budding yeasts but forming no asci.

Genus I. *Torula*.

Spherical cells, usually, form no ascospores, reproduce by budding; the cells are also smaller than many other yeasts; low fermenting ability.

Genus II. *Mycoderma*.

Film forming, usually elongated cells, develop in the presence of air; no ascospores; reproduce by budding.

INDUSTRIAL YEASTS

The yeast plant is a wonderfully active and useful organism. Each cell of certain species is a manufacturing plant of marvelous efficiency and productivity. By means of fermentations brought about by yeast cells, man, for ages, has brought about desired changes in foods and beverages. In most of these cases, he has not known until recently just what was responsible for the changes, for knowledge with regard to yeasts was not forthcoming until the researches of Pasteur, Hansen, and others.

Industrial Classification of Yeasts.—In the various fermentations, yeasts are classified into top, bottom and distillery yeasts. The groups are not sharply separated nor defined.

Top Yeasts.—Top yeasts are those which carry on fermentation in the upper layers of the medium. They ferment with a vigorous foam or froth, spoken of as "head" in fermentation industries, which carries many of the cells to the top. Such a fermentation is termed a top-fermentation. Most of the yeasts in pressed yeast are top yeasts. Top yeast, in general, remain attached, giving masses of cells, while bottom yeasts separate. The top yeasts produce more alcohol than the bottom yeasts. After active fermentation has stopped these species may settle to the bottom. Many of the top-fermentation yeasts are unable to

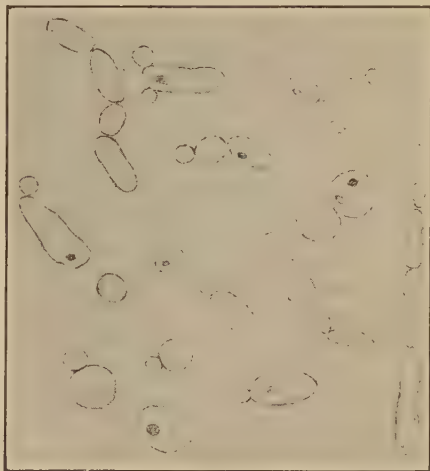


FIG. 87.—Showing Budding of Yeast Cells.
(After Wehmer.)

ferment melibiose. Top fermentations induced by top yeasts are accompanied by large quantities of foam (head).'

Bottom Yeasts.—Bottom yeasts grow and ferment down in the liquid or even at the very bottom of the substrate. They produce lower amounts of alcohol. Bottom yeasts contain an enzyme, melibiase, which allows this species to ferment melibiose.

Distillery Yeasts.—These are yeasts which are used for the making of

industrial alcohol and the distilled beverages. They are distinguished from the other yeasts by a marked fermentation ability and alcohol tolerance.

Preservation of Yeast (Compressed Yeast).

There is scarcely a hamlet which to-day does not have its supply of fresh pressed yeast. Two methods are used for its manufacture. The oldest method is known as the Vienna process. It may be outlined as follows:

Raw Materials.—Most of the effort at propagating yeast is expended for a suitable medium upon which to grow it. This is generally made from a mixture of grains—corn, rye, and barley malt, according to the formula of the manufacturer. The grains are at first

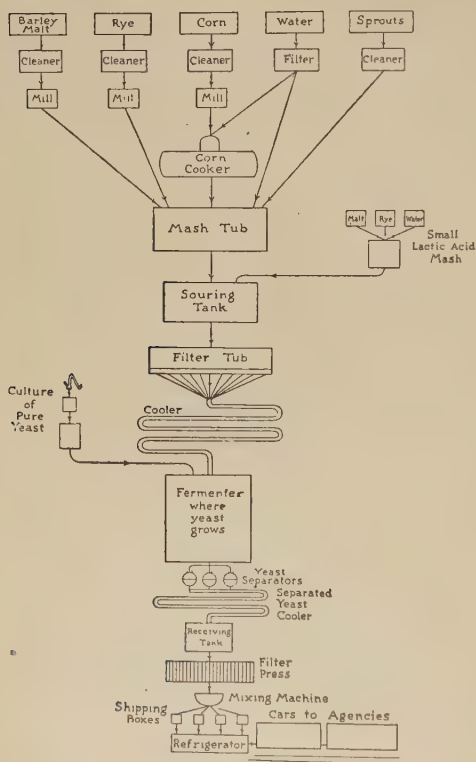


FIG. 88.—Showing the Several Steps in the Preparation of Compressed Yeast. (After Fleischmann Co.)

thoroughly cleaned and stored; when needed they are ground separately.

Preparation of the Mash.—The flours secured from grinding the grains are blended and soaked in clean water. This softens the starch particles and extracts various food materials which are necessary for the yeast plant. Malt is also added to change the starch to sugar. This gives a sweet mash rich in food materials desired by yeasts.

Souring or Lactic Process.—Pure cultures of lactic acid bacteria are next added to the mash. These organisms form lactic acid in the mash. This acid, as in many other cases, counteracts putrefaction and causes the mash to keep.

Filtration of the Mash.—When the mash has soured sufficiently it is filtered, a clear straw-colored liquor known as “wort” being secured. It is then heated to a high temperature to sterilize it.

Fermentation.—This clear wort is the medium upon which the yeast is propagated. It is carried in pipes to the fermenters where it is inoculated and kept at the proper temperature for the growth of the yeast. While the fermentation is going on air may be blown through the wort. This removes the CO_2 and causes a greater yield of yeast cells.

Harvesting of the Yeast.—At the end of the fermentation, the wort is filtered or centrifuged to remove the yeast cells. They are collected as a thick cream and finally carefully dried further in filter presses. The cells from the presses are mixed with about 5 per cent of starch, pressed into cakes and shipped to centers where they are cut up for sale.

Instead of the laborious preparation of wort outlined above some yeast manufacturers have introduced a sort of synthetic wort made from sugar or molasses and ammonium sulfate. This is also aerated while the yeast is growing in it. Aeration removes the gaseous products of yeast metabolism as well as stimulates cell division mechanically.

YEAST-LIKE FUNGI

Under the name yeast-like fungi are grouped a number of organisms which are like the yeasts in many respects such as appearance of growth on laboratory media, shape of cell, etc., but still lack the botanical characters to place them with the yeasts.

THERAPEUTIC USE OF YEAST

Since very early times yeasts of different types have been used for the treatment of disease and infections. It has been stated that the monks used yeast for the treatment of several diseases. Hippocrates advised the use of yeast for the treatment of leucorrhea. Since these times there seems to have been a continuous interest in yeast as a therapeutic agent. The present-day interest in the use of yeast in this respect began, perhaps, with a paper by Hawk² and his colleagues in which they

² Hawk, P. B., *et al.* The Use of Bakers' Yeast in Diseases of the Skin and of the Gastro Intestinal Tract. Jour. Amer. Med. Assn., 69 (1917).

reported the use of yeast with beneficial results in infections of the skin and constipation. These experiments were not well controlled, and before the conclusions of Hawk and his colleagues are extensively applied further work should be done. It seems quite well established that the ingestion of bakers' yeast will alleviate constipation. Whether a cure results has not been satisfactorily established. Modern medical science dislikes the continued use of a drug or other agent to relieve constipation. It strives, on the other hand, to correct the cause. One may reasonably question the advisability of prolonged use of yeast as a curative agent. Very little, if any, satisfactory evidence exists to indicate the absolute harmlessness of constantly overloading the intestinal tract with living yeast cells.

PATHOGENIC YEASTS

While some of the yeasts play a very important rôle in certain industries, there are also species which cause very serious infections in animals. Far less is known about these forms than about the bacteria which cause disease. It would be out of place to enumerate at great length the infections and species which cause these infections, in man and animals.

Thrush.—This is an infection of the mouth and throat; it is especially common in babies. The identification of the etiologic agent is confused. It was first described under the name of *Oidium albicans* by Robin. Later Reiss called it *Saccharomyces albicans* which was probably wrong. Now it is known under the names *Endomyces albicans* and *Monilia albicans*. This same fungus is apparently capable of causing other infections, also, in the animal body. Formerly the disease was thought to be mainly a tropical infection, but more and more reports are appearing in our literature indicating its prevalence in the temperate zones.

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CHAPTER IX

THE PROTOZOA

This is a group of animals of interest to bacteriologists in that they are single-celled microorganisms structurally like many of the bacteria. On this account some biologists believe that the bacteria should be classed with the animals. A protozoon is a low animal form, single-celled, composed of protoplasm and containing a nucleus. The protozoa constitute the first phylum of the animals but possess many characteristics which connect them with plant forms. There are good reasons, if one wishes to exercise his desire for controversy, for placing the protozoa in the plant or animal kingdom as over the location of the bacteria. All protozoa are small and most of them require a microscope to make them visible.

Morphology of the Protozoa.—Protozoa are single-celled forms of life which show great variation in size and shape. A similar variation in size and shape exists among the protozoa as exists in the bacteria. Some of the protozoa are colonial and live together in groups or masses. These masses are often times visible without a microscope, whereas the individual members of these colonies are not.

Cytoplasm.—The cytoplasm of a protozoon is not greatly different from the cytoplasm of other cells. It is colloidal in nature and quite fluid. It is separable into two zones, the *ectoplasm* and the *endoplasm*. The ectoplasm is an outer, denser layer about the cell while the endoplasm is a thinner less dense zone at the center of the cell.

Nucleus.—The nucleus plays an important rôle in reproduction of the protozoa. It is, also, important in the vital phenomena of the cell. If a protozoan cell is cut into two parts so that one part has the entire unharmed nucleus, that part will regenerate itself. The portion without the nucleus will not. The nucleus is demonstrable with nuclear stains; it is, also, more refractive than the rest of the cell. The presence of a nucleus in protozoa should be of interest to students of bacteriology in relation to the controversy over the presence of a nucleus in bacterial cells. A protozoon or yeast cell without a nucleus would be regarded as impossible. These cells are large enough for study and many purposes have been given the nucleus in the life cycles of these organisms. It is apparent, then, that as soon as we reach cells which are large enough

for convenient study and examination, the nucleus is admitted to have important functions.

Centrosome.—Centrosomes have not been uniformly found in the cells of all protozoa. In those forms of life where its presence has been established, it is said to play a rôle in reproduction and division of the nucleus.

Nutrition of the Protozoa.—Protozoa are able to use both liquid and solid food. Most of the saprophytic forms use solid food or at least are able to convert solid food into a condition in which it may be used. The liquid food passes through the cell wall by osmosis. The waste products are excreted in the same manner with those forms which do not possess definite excretory organs. Some protozoa possess contractile vacuoles which excrete material.

Reproduction of Protozoa.—The evidence that reproduction of the cell is about to occur is first of all seen in the nucleus. The nucleus divides into two nuclei; this is followed by division of the cell. This process is called binary fission, a method of reproduction also used by the yeasts.

Conjugation¹ of cells may occur among the protozoa just as it has been observed for yeasts and perhaps the bacteria. Hertwig stated that sometimes this did not result in fertilization since only the protoplasm fused; the nuclei did not. At other times there is a fusion of the nuclei. When such copulating cells are equal in size they are called isogametes; when they differ in size and small cells, the males, are called microgametes; the larger female cells are called macrogametes. The small male microgametes fertilize the larger female macrogametes.

Protozoa also divide by fission in about the same manner as the yeasts. A small bud appears on the side of a larger cell; this bud grows in size until it finally breaks away from the parent cell to exist as a separate unit. This is generally regarded as an asexual method of reproduction.

Sporulation.—Among many forms of life resistant stages are found in the life cycles. These are spoken of as spores, conidia, ascospores, etc. Similar stages are also found in the protozoa. Certain cells seem to be able to become encysted by forming a resistant wall about themselves. This process is not unlike the formation of the so-called arthrospores among the bacteria. While the cell is in the encysted stage, it is resistant to unfavorable conditions and may be disseminated by wind and water. When favorable conditions occur again it germinates into an actively growing cell.

¹ Copulation is a temporary union between two gametes; conjugation is a permanent union.

Classification of the Protozoa.—As with bacteria, morphological and physiological characteristics are used for classification. With the protozoa, however, the most satisfactory classifications have been constructed with the use of morphological characteristics. The following classification is adapted from one by Calkins.

PROTOZOA

Unicellular animal organisms which reproduce by division or spore formation; solitary or united in colonies; free living or parasitic.

Sarcodina: Protozoa with changeable protoplasmic processes or pseudopodia; naked or shell bearing; reproduce by fission and spore formation. Genus parasitic for man *Endameba*.

Mastigophora: Protozoa with flagella; many of them possess mouth, nucleus and contractile vacuole. Genus parasitic for man: *Trypanosoma*.

Infusoria: Protozoa with cilia by which locomotion is accomplished; reproduction both by budding and by fission; possess macronucleus and micronucleus. Genus parasitic for man: *Balantidium*.

Sporozoa: Protozoa without motile organs; reproduction by sporulation; always parasitic. Genera parasitic for man *Coccidium*, *Piroplasma* and *Plasmodium*.

PATHOGENIC PROTOZOA

Among the protozoa are species which cause serious infections in man and animals. A few of the more common ones will be discussed.

Amebic Dysentery.—This is a disease restricted usually to tropical climates although cases have been discovered in the temperate climates. It is caused by *Endameba histolytica*. The parasite causes the formation of large abscesses or ulcers by penetrating the intestinal walls. It may also penetrate the body tissues and cause the appearance of abscesses in the internal organs. Such abscesses like those which occur in the intestines contain large numbers of protozoa cells.

Sleeping Sickness.—This is another tropical infection caused by *Trypanosoma gambiense*. This parasite is transmitted by means of the bite of the tsetse-fly. It has been shown that the blood of infected animals contains the above-mentioned trypanosome. The parasite remains in the fly for a long time, 12 to 15 weeks or longer. The fly *Glossina palpalis* seems to be the one which is most liable to disseminate the disease. This seems to be the case since in localities where the fly is not found there are no cases of sleeping sickness. The typical symptoms are caused by the Trypanosome entering the cerebrospinal fluid. The parasite may be present in the blood for some time before it enters the nervous system. The symptoms, in general, are fever and pro-

nounced somnolence usually followed by death. The characteristics of the parasite may be secured from various reference texts on protozoology and parasitology.

Malaria.—The name malaria covers several closely similar communicable fevers to which man is subject. There are several parasites all protozoan in nature which are grouped under *Sporozoa*, making the genus *Plasmodium*. Malaria is an endemic disease in certain sections of our country. The mosquito is the only means by which man may be infected. The several varieties of malarial parasites make it possible to classify the malarial fevers as follows:

1. Tertian fever caused by *Plasmodium vivax*.
2. Quartian fever caused by *Plasmodium malariae*.
3. Aestivo-Autumnal fever caused by *Plasmodium falciparum*.

The separation and identification of these parasites is not a settled matter.

Malaria cannot be spread by all mosquitoes. Those which spread the disease are *Anopheles*. There is but one way in which malaria may be contracted and that is by means of the bites of certain mosquitoes. Consequently, to have malaria three factors are involved: malarial parasites, healthy men and *Anopheles* mosquitoes. Only the female bites and if she feeds on a person suffering from malaria, she imbibes some of the parasites; when she feeds on a healthy person, some of the parasites are injected into him. These parasites enter the blood corpuscles where they grow larger and larger and finally reproduce to make a number of progeny. The blood corpuscle in which this occurs then breaks up and each of these parasites repeats the process in another corpuscle. The time required for this process to occur varies with the parasite. The *tertian* required forty-eight hours, the *quartian* seventy-two hours, and the *aestivo-autumnal* from twenty-four to forty-eight hours. When the blood corpuscles break up to liberate the parasites, a poison is also liberated which causes the chill and fever.

The historical period when the etiology of malaria was being worked out is just as interesting as that of other branches of medical science. Years of investigation were required before the mosquito was known to be so important. A great number of investigators had a part in proving this. A complete discussion of this would require too much space. Sir Ronald Ross of the Indian Medical Service probably was the first to show that the *Anopheles* mosquito spread the etiologic agents of malaria. Among the investigations which confirmed this was a convincing experiment carried out by Drs. Sanborn and Low. They went to a region which was renowned for its malaria, the Roman Campagna.

They lived in a hut carefully screened and refrained from going out at night when the malarial mosquito bites. They did not take the disease. They also sent some mosquitoes which had bitten persons suffering from the disease to London where their bites caused the disease in healthy subjects. Other experiments just as convincing made out the case against the mosquito.

The important facts about malaria have been summarized as follows by a committee of prominent sanitarians.

MALARIA

1. *Infectious Agent*.—The several species of malarial organisms—*Plasmodium vivax*, (tertian); *Plasmodium malaria* (quartian); *Laverania falciparum* (aetiv-autumnal).

2. *Source of Infection*.—The blood of an infected individual.

3. *Mode of Transmission*.—By bite of the infected Anopheles mosquitoes. The mosquito is infected by biting an individual suffering from acute or chronic malaria.

The parasite develops in the body of the mosquito for from ten to fourteen days after which time the sporozites appear in the salivary glands.

4. *Incubation Period*.—Varies with the type of species of infecting organism and the amount of infection; usually fourteen days in the tertian variety.

5. *Period of Communicability*.—As long as the malaria organism exists in the blood.

6. *Methods of Control*.—

(A) The infected individual and his environment.

1. Recognition of the disease—Clinical symptoms always to be confirmed by microscopical examination of the blood. Repeated examinations may be necessary.

2. Isolation—None except protection of the patient from approach of mosquitoes by screening his bed or room or house, until his blood is rendered free from malarial parasites by thorough treatment with quinine.

3. Immunization—None. The administration of prophylactic doses of quinine should be insisted upon for those constantly exposed to infection and unable to protect themselves against Anopheles mosquitoes.

4. Quarantine—None.

5. Concurrent disinfection—None. Destruction of Anopheles mosquitoes in the sickroom.

6. Terminal disinfection—None. Destruction of Anopheles mosquitoes in the sick room.

(B) General measures:

1. Employment of known measures for destroying larvae of anophelines and the eradication of breeding places of such mosquitoes.

2. Blood examination of persons living in infected centers to determine the incidence of infection.

3. Screening sleeping and living quarters; use of mosquito nets.

4. Killing mosquitoes in living quarters.

Texas Fever.—This is a disease of cattle. It is spread by the bite of a certain tick which, in turn, has fed on the blood of an infected animal. The parasite belongs to the genus *Piroplasma*. The disease is fought by dipping the cattle in arsenic washes and by grazing them in pastures not harboring ticks.

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CHAPTER X

ACTION OF PHYSICAL AGENTS ON BACTERIA

The fact that bacteria are susceptible to injury by many of the physical agents gives us methods for coping with the undesirable and controlling the desirable species. Since the bodies of bacteria are composed of protoplasm, and since protoplasm is a delicately equilibrated substance, their life processes may be easily upset. Such agents as heat, light, etc., exert a detrimental effect on bacteria. Some of the physical agents, such as temperature, moisture, etc., are also important in the the propagation of bacteria. It is necessary to maintain a proper temperature, for instance, to secure a good growth of microorganisms.

LIGHT

Light is detrimental to bacteria. This may be easily shown by experiments in the laboratory. Considerable work has been carried out to determine how light exerts its harmful effect. One of the earliest explanations indicated that certain harmful chemical substances were formed in the medium by light. These were suggested to be ozone, hydrogen peroxide, and the like. These agents are disinfectants, whatever their origin. Later, data were collected which seemed to refute this explanation of the toxic action of light. Another explanation suggested that the light acted directly on the protoplasm of the cells causing a coagulation of protein incompatible with the life of the cell. Another fact interesting to contemplate in this connection is the absence of chlorophyll in bacterial cells. This material gives the cells which contain it the ability to use the energy in light for the construction of large molecules from simpler ones. Chlorophyll-containing organisms are thus able to prevent an accumulation of this energy. The non-chlorophyll microorganisms, being devoid of this substance, may be unable to prevent this and consequently are more sensitive to light.

Sunlight.—Strong sunlight is quite destructive to bacteria when it is allowed to reach them directly. It shows a similar destructive action on cells of the human body as evidenced by sunburn and tan. It has been advised as an hygienic agent of no mean ability. This ability has

probably been over-estimated since the bacteria may be protected from its action by bits of organic matter, etc. More reliable agents are available with which to destroy bacteria. Early workers found that diffuse daylight inhibited the growth of bacteria while direct sunlight killed them. The destructive action of sunlight has been offered as an explanation of the decrease of bacteria in natural bodies of water. Its activity in this case depends on several factors, such as turbidity, reaction, chemical content, etc.

Buchner in 1897 carried out an experiment with *Eberthelli typhi* (*Bacterium typhosum*) which showed the destructive effect of light on bacteria. He heavily seeded a culture dish containing food materials.



FIG. 89.—Showing the Influence of Sunlight on Bacteria. (After Buchner.)

On the bottom of the dish was pasted the word TYPHUS in large block letters of black paper. The dish was then exposed to the sun for an hour and a half after which it was incubated. After the paper letters had been removed their location was indicated by whitish growth where the opaque paper had prevented the sun's rays from killing the bacteria.

Effect of Different Colors of the Spectrum.—Numerous investigators have studied the various parts of the spectrum for their effects on bacteria. Since they worked with different organisms under different conditions, their data ought not to be compared too minutely. We know, however, that the colors in the spectrum become more toxic from

the red to the violet end. The ultra-violet rays are very germicidal. Ward, the great English botanist, studied the question and reported that from the red to the green, the colors were without effect; beyond the green the toxicity increased to fall away again in the violet and ultra-violet.

The various parts of the spectrum are characterized by their specific wave lengths. That portion of the spectrum with wave lengths of sufficient magnitude to be visible to the human eye is known as the visible spectrum (from the red to the blue); those portions which cannot be seen by the human eye but which are demonstrable by physical instruments, are spoken of as the ultra-violet on the blue end of the spectrum and infra-red on the red end. The ultra-violet, as the name indicates (beyond the violet), is that invisible ray just beyond the blue. The unit of light wave length must be so small that ordinary units of measurement would be too cumbersome to use. One would be using such long decimals that there would be greater possibility for error and much time would be lost. Consequently, physicists have done what the microscopists had to do, created a new unit of measurement. This is called the Ångström unit and is one ten-millionth of a millimeter in length. Besides the Ångström unit some investigators use the unit of measurement millimicron ($\mu\mu$). A millimicron is one-millionth of a millimeter. For converting millimicrons into Ångstrom units multiply by 10 or add a cipher.

Ultra-violet Light.—This portion of the spectrum which is invisible to the human eye on account of the very short wave length, may be singled out for special discussion. Recent experimental work indicates that it is endowed with exceptional properties. It is often spoken of as the quartz ray since it is emitted from a quartz lamp. This portion of the spectrum is quite germicidal for bacteria. Once this had been established, many attempts were made to apply the ultra-violet ray for killing undesirable bacteria and the cure of disease. Attempts were made to sterilize water. While under some conditions it was found to be a satisfactory germicide, it was too expensive when compared with other available methods. It was suggested for the pasteurization of milk, but on account of the opacity of milk it had to be given up. During the War it was suggested for the sterilization of bacterins. One difficulty in using the ultra-violet ray is the fact that expensive quartz apparatus must be used since the rays do not pass through ordinary glass.

X-Rays.—This form of energy is very toxic to living protoplasm. It is used for treating some of the diseases caused by thread fungi such as ring-worm, itch, etc. These fungi, because of a rich filamentous growth,

penetrate into the deeper layers of the skin where they are reached with difficulty by fungicides and disinfectants. In using the X-ray, it seems to be difficult to prevent harming the tissue. Some investigators have stated that it is possible to destroy the tissue cells without destroying the bacteria for which the X-rays were used.

TEMPERATURE

This is indeed an important physical agent for all forms of life. Many organisms carry on their life processes at a single temperature. Higher animals are separated into two groups on this basis, the cold-blooded animals and the warm-blooded animals. Microorganisms are much more susceptible to high temperatures than to low temperatures although the latter are probably more harmful than is ordinarily believed. Here again it is well to point out that our data result from the use of a great many cells in experiments and not from observations on one cell.

Classification of Bacteria According to Temperature.—The reaction of bacteria to temperature gives us a means of separation into groups. This is another illustration of the physiological grouping of bacteria. These groups, of course, do not have well-defined limits and consequently grade into one another, but, they are convenient for designating organisms having like temperature relations.

Thermophilic Bacteria.—These are bacteria with high temperature relations. The optimum may be placed between 55° – 60° C., the maximum at 70° – 75° C., and the minimum at perhaps 45° C., although this is arbitrary. Some authors speak of obligate or strict thermophiles as those which constantly require a high temperature for growth, and facultative thermophiles as those able to grow at high temperatures and also at temperatures approaching the optimum of the mesophilic bacteria. The thermophilic bacteria are interesting since they grow best at a temperature which is lethal for many other types of protoplasm. Many of the proteins are coagulated at 55° – 60° C.

Psychrophilic Bacteria.—As the name indicates, these are cold-loving bacteria. They grow well at low temperatures. Their optimum might be placed around 5° – 10° C., with a minimum at just above 0° , or just where the medium in which they are remains liquid. These forms are significant in the preservation of foods in cold storage.

Mesophilic Bacteria.—Between the thermophilic and psychrophilic groups fall a great many species, the mesophilic bacteria, with an optimum about 30° – 37° C. In this group are the pathogenic forms, those causing disease in man and other animals, and the soil or water organisms.

It is difficult to give the temperature limits for these groups of bacteria separated on the basis of temperature. Fischer stated them as follows:

	Minimum	Optimum	Maximum	Types or Species
	Deg. C.	Deg. C.	Deg. C.	
Psychrophilic	0	15-20	30	Many water bacteria
Mesophilic	15-25	37	43	Pathogenic and other bacteria
Thermophilic	25-45	50-55	85	Spore-forming bacteria from soil, water, thermal springs, etc.

Temperature Characteristics of Individual Organisms.—Like the other characteristics of pure cultures, the temperature relations are equally important in describing an organism. The following three temperature characteristics are determinable:

Maximum Temperature.—This is usually defined as the highest temperature at which an organism may live and carry on any of its life processes. There may be a different maximum temperature for every function. Thus, the maximum temperature for growth is quite different from that for fermentation. When maximum temperatures are given for an organism they usually refer to growth. At maximum temperatures the cell is being driven at a rate beyond that which is efficient. There is probably little opportunity for cell repair, at least for the cell repair which is necessary to keep the cell in good shape. At the maximum temperature, katabolism exceeds anabolism. This is also demonstrable in higher animals. High temperatures in the summer lead to general malaise, etc.

Minimum Temperature.—This is the lowest temperature at which some of the functions are carried on. The minimum temperature, of course, would have to be above the freezing-point. At its minimum temperature a cell is running very slowly in about the same manner that an automobile engine "idles" when the supply of fuel is reduced. At lower temperatures there is less wear on a cell and it will live much longer. This is well illustrated in Fig. 90. The curves show the histories of cultures of the same organism grown at 37° C., 27° C. and 17° C. The organism was *Staphylococcus aureus*. The culture propagated at 17° C. gave higher numbers of bacteria and maintained higher numbers for a longer period. At 37° C., the culture gave its maximum growth in a shorter time but the cells wore themselves out more quickly.

Effect of Freezing on Bacteria.—In their reaction to freezing temperatures, bacteria differ in some respects from plants and higher animals. Bacterial cells are more resistant to freezing than cells of higher plants. Under the subject of thermal death-point, it may be pointed out that in connection with heat the time factor is important; it is just as important in relation to extreme cold or any other harmful agent. Bacteria have been exposed to the temperature of liquid air ($-200^{\circ}\text{C}.$) and have survived. This does not mean that there is not a loss in numbers. The number of cells decreases very rapidly and the condition of complete sterilization depends on the number of cells with which the experiment was started and other factors. Thomas found that the major portion of cells of *Eberthella typhi* (*Bacterium typhosum*) was destroyed by freezing in about a week. It has also been found that the bacteria in ice

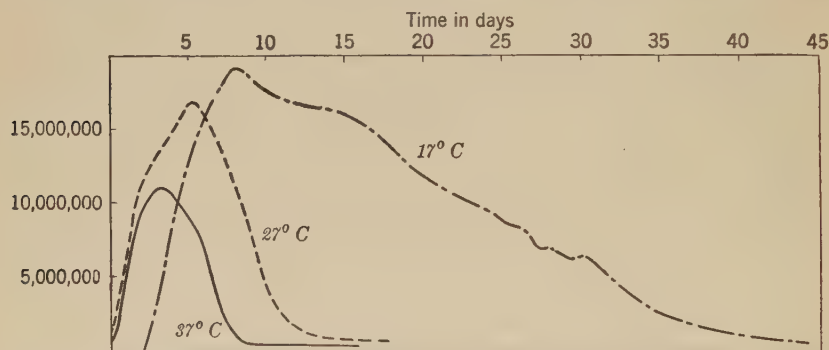


FIG. 90.—Showing the Results of Different Temperatures of Incubation on Broth Cultures of the Same Organism. (After Graham-Smith.)

cream at first decrease rapidly in numbers, after which there is a slower death rate. The statements that bacteria are not killed by freezing which appear in so many texts rest, perhaps, on faulty technic or false logic. The fact that living cells may be shown to be present in suspensions which have been frozen, is no indication whatever that freezing is without effect. Some few cells may be more resistant than others and require longer freezing for destruction.

The medium in which the organisms are frozen undoubtedly has influence. Perhaps the hydrogen-ion concentration is just as important in this connection as it is in the destruction of bacteria by heat. The physical condition of the organism would also influence its reaction to cold.

When bacteria are frozen in some medium, the water necessary for their life processes is not available. It is in a crystalline state and not

available for the cell. In this sense, then, the cells are in a dry environment, and death by freezing may be comparable to death by drying.

This topic may be left with the question, why are bacteria so much more resistant to cold than the cells of higher plants and animals? Some plant cells may be injured by the first heavy frost in autumn; certain animal cells are injured by extreme cold. Bacterial cells seem to be more resistant. When suspensions of bacterial or yeast cells are frozen, there is a high death rate at first, but, a point is soon reached where the living cells remain almost constant in numbers. One may wonder about the internal conditions of a cell which is not killed by prolonged sojourn at temperatures below zero.

Optimum Temperature.—This is the best temperature for the function of the organism under discussion. The optimum temperature for growth may be quite different from that for spore formation, fermentation, death, etc. Statements about optimum temperatures usually apply to growth or multiplication, although the functions of the organism should be mentioned. At the optimum temperature the cell is probably working to best advantage and its machinery is working at such a rate that there is an equilibrium between anabolism and katabolism.

Thermal Death-point of an Organism.—This is that temperature which will kill an organism under certain arbitrarily selected conditions. It is impossible to define the term in a few words. Possibly definition should not be attempted. There is no one place on the temperature scale where all of the cells in a suspension die instantaneously. Therefore when reporting data from attempts to determine the reaction of an organism to lethal temperatures, the time and temperatures should be stated. There is probably no such characteristic as a thermal-death-point. When studying the reaction of an organism to heat the following factors have to be kept in mind.

1. *The Time.*—Temperature and time cannot be separated and when but one of these factors is being studied the other must be taken into consideration. There is an indirect relation between these two factors. To accomplish certain results we may use a higher temperature for a shorter time or we may use a lower temperature for a longer time. This is true also for chemical reactions. The chemist brings about reactions with a Bunsen burner in the laboratory that would require years to accomplish at room temperature.

The relation of time and temperature is well illustrated by two processes for the pasteurization of milk that have been used at various times. One was spoken of as the *flash* process in which the milk was heated to about 90° C. for one or two minutes, and the other, in use at present, as the *continuous* process in which a temperature of 62.5° C. is

maintained for thirty minutes. In the former method the temperature was high and the time short while in the latter the temperature is lower, necessitating a longer heating period if the same killing effects on bacteria are to be obtained.

2. *Number of Cells.*—It is well known from chemistry that the concentration of reacting substances has much to do with the speed of the reaction. Consequently, in determining thermal death-points, where we are killing bacteria with moist heat for instance, the same information is pertinent. The larger the number of cells present to be killed the longer it will take to attain sterile conditions. It is also probably true that the greater the number of cells, the greater the possibility of securing very resistant cells which will be able to endure much greater amounts of heat than ordinary cells.

3. *Amount of Water Present.*—This is one of the most important essentials in thermal death-point studies. It is known that water is necessary for the coagulation of proteins and the destruction of bacteria is probably due to this change. We have learned before that the materials which go to make up protoplasm are colloidal in nature. When such materials are heated a "gel" is formed. This may be incompatible with the continued normal activities of the cell. Frost and McCampbell in their text-book on bacteriology present the following table. This has much interest, not only in this connection, but also in explaining why such widely different temperatures are used in dry and moist heat sterilization.

Egg albumen + 50 per cent of water coagulates at 56° C.
Egg albumen + 25 per cent of water coagulates at 74°–80° C.
Egg albumen + 18 per cent of water coagulates at 80°–90° C.
Egg albumen + 6 per cent of water coagulates at 145° C.
Egg albumen + 0 per cent of water coagulates at 160°–170° C.

4. *The Age of the Cells.*—Old cells are said to be more easily killed. Bacteriologists appreciate the necessity of using comparatively young cells, hence 24- and 48-hour-old cultures are used in experimental investigations unless older cultures are desired on account of some special property. Old cultures may contain cells which are worn out and devitalized. On the other hand, cells which are very young might be more susceptible to heat since they might have to mature somewhat before showing a marked resistance to heat.

5. *Reaction of the Medium.*—For a long time it has been known that the reaction, or hydrogen-ion concentration of the medium greatly influences the rate of death. Those who preserve foods by canning know that acid foods such as strawberries, cherries, and rhubarb are

preserved with relatively greater ease than such foods as peas and corn. The latter are nearer neutral in reaction. Since the hydrogen-ion concentration is so important, it is necessary to control it in thermal death-point studies.

6. *The Type of Organism.*—In this respect also, bacteria differ from one another. The same observations may be made on the higher animals for they differ in their resistance to and tolerance of heat. It has been stated before, for instance, that the thermophilic bacteria have their optimum at a temperature which approaches the maximum for other forms. The bacteria show the same variation in resistance to heat that is shown among higher animals. Some human beings are able to tolerate hotter climates than others.

7. *The Presence of Spores.*—The ability to form spores gives the organism more resistance to heat. Theoretically, then, there may be a thermal death-point for the vegetative protoplasm and one for the sporoplasm. Practically, however, the distinction, although made by some bacteriologists, is of little importance. Most spores are far more resistant to heat than are vegetative cells.

Thermal death-point studies are important in several phases of applied bacteriology. Such information is desired about each separate organism in order for it to stand apart from others as a species. In the canning industry, information is required relative to the temperature relations of bacteria which spoil food packed in cans. Such bacteria are usually isolated in pure culture, suspended in the liquor of the food from which they were isolated, and subjected to tests to determine their heat resistance. Thus is developed information about the amount of heat necessary to destroy the organisms in the canned food materials.

Relation of Temperature to Chemical Changes.—What effect does temperature have on the chemical changes brought about by bacteria? It is known that as the temperature is raised the speed of chemical reactions is increased. There is a limit, of course, to the general application of this statement, for other factors have to be considered. For instance, if the temperature reaches a certain point the reacting substances may be changed. So with chemical reactions brought about by bacteria, temperature may speed up their reactions only below their maximum temperature above which they are killed. Investigators have shown that the yield of certain products of bacterial action increases as the temperature is lowered. This was explained by stating that at the higher temperatures, the products might have a greater action on the bacterial cells. We may also assume that above the optimum temperature, at least, the cell is being driven too fast by the higher temperature; it wears itself out more quickly because there is no opportunity for repair.

At the lower temperature, however, the cell works more economically because there is opportunity for repair and a better equilibration of cell mechanisms. The thermophilic bacteria are especially suited to studies of this nature. Their optimum temperature is near 55°C . Some of them will grow as low as 37°C . One species, which decomposes cellulose, is found to decompose forty times as much at 55°C . as at 37°C . At 55°C . these organisms are working at a more rapid rate since they are driven by the high temperature.

The stimulating effect of increased temperatures is just as evident with some of the higher forms of life. Biologists have found that more than twice as many generations of flies (*Drosophila*) are formed at 27°C . than at 17°C .

Destructive Action of High Temperatures (Sterilization by Heat).—Heat is one of the cheapest and best agents for the destruction of cells of microorganisms. It is used in many of the great industries for the killing of bacteria and preservation of food materials. The bacteriologist has great need for heat since his work involves the separation of a species of microorganism from others and its propagation in pure culture. To do this all of the food substances (media) and apparatus must be sterile (free from all forms of living matter). This is known as *sterilization*, the absence of all forms of life in or upon materials or objects. In contrast with the term sterilization we use the term *disinfection*, defined as the destruction of disease-producing organisms only. The distinction between the terms is close and, perhaps, quite unnecessary. Obviously, all methods of sterilization are methods of disinfection but methods of disinfection are not necessarily methods of sterilization. Under many conditions they probably are.

We will now discuss some of the procedures for sterilizing.

I. Dry Heat.—The term dry in this connection implies the absence of a great amount of water about the cells. There is, of course, the usual amount in the protoplasm. On account of a lower amount of water present, a higher temperature must be used. The data from Frost and McCampbell on page 190 show that with zero per cent of water, egg albumen coagulated between 160° and 170°C . This indicates that a temperature above this must be used in dry-heat methods of sterilization.

1. *Incineration.*—This is the most satisfactory method for getting rid of undesirable bacteria but has very decided limitations. It is used for the destruction of dressings, etc., from wounds and infections. Obviously it cannot be used for materials which must be preserved for future use; some of the other methods must be employed.

2. *Heating in flame.*—This method has been spoken of as the incidental method. It is a very useful method and is used by bacteriolo-

gists for sterilizing the needles and loops with which bacteria are transferred from one tube to another. The heating must be even and the cooling slow. When needles and loops are sterilized in the free flame, care must be exercised to avoid spattering of any material on them and a dissemination of infectious matter.

3. *Hot-air Oven*.—The hot-air oven used in the laboratory for sterilization is quite similar in construction with that used in the home for baking. It consists of a sheet-iron box with a gas burner either under it or about the sides. Many different types are available of which the Lautenschläger type is most common. Some hot-air ovens are so con-

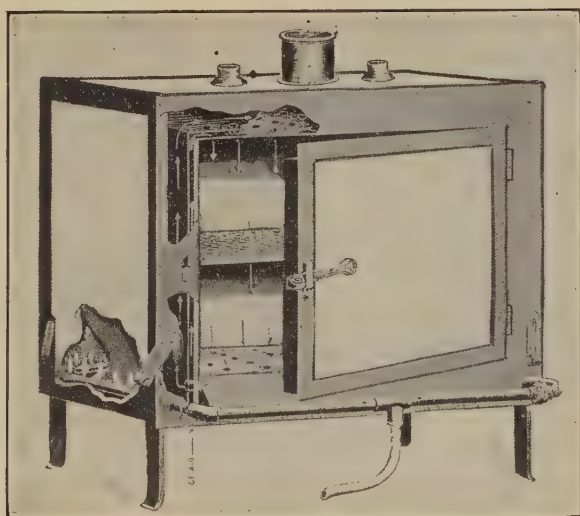


FIG. 91.—Hot-air Sterilizer, Lautenschläger Type.

structed that a high temperature is maintained with the heat turned off for a long time after it has once been raised to the desired point. This is accomplished by as thorough insulation as possible.

II. *Moist Heat*.—The presence of adequate water allows disinfection to go on much more readily. Consequently, a lower temperature may be used than in the dry-heat method. Water is necessary for the coagulation of proteins and the more water there is, the more easily is disinfection by heat accomplished. Besides its influence in the coagulation of proteins, water may also assist in carrying the heat units into the cell. This is nicely shown by an illustration from the canning industry. Foods, such as sweet potatoes, roast beef, etc., packed solidly into cans are sterilized with more difficulty than foods with free liquid such

as peas, beans, etc. The free liquor in the case of beans, peas, etc., carries the heat into the can by convection.

1. *Boiling*.—Proteins are coagulated by boiling, which may also explain sterilization by moist heat. The length of boiling required depends on a number of factors. Practically all the factors influencing thermal death-point determinations also influence boiling as a sterilizing procedure. Boiling is a very convenient method for sterilizing such things as tableware, linen, etc. Before instruments with cutting edges are placed in water, the water should be boiled to rid it of the dissolved oxygen which might attack the sharp edges and cause rusting. By

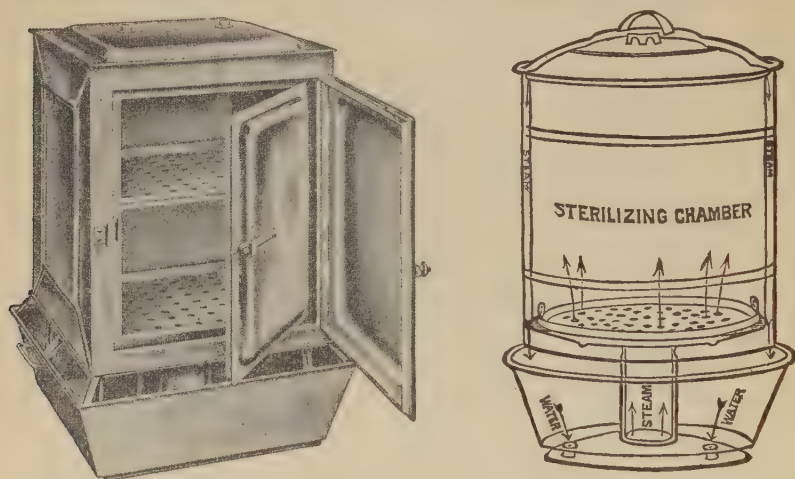


FIG. 92.—Showing Free Flowing Steam Sterilizers.

A, Boston Board of Health Type. B, Cross Section of a Similar Pattern.

many, boiling is considered an infallible hygienic procedure, but it has certain limitations. It has been suggested by food experts in the government laboratories that all canned foods be boiled before use in order to give greater certainty of freedom from illnesses which may be caused by such foods. They have pointed out that canned foods which have supported bacterial growth might be heavily impregnated with gas and that such foods would give the appearance of boiling much below the true boiling-point. Another influencing factor is altitude. In certain sections of the country, this factor has great influence on the boiling-point.

Boiling water and soap are probably the best cleansing agents available. A committee of prominent sanitarians in America recommended them in place of fumigation after cases of communicable diseases in the

home. Boiling water and soap suds are undoubtedly more destructive to bacteria than some of the supposedly bactericidal procedures used in terminal disinfection.

2. *Free Flowing Steam*.—This is steam which is free and consequently at a temperature of 100° C. Since the temperature is constant, the only variable element in the use of this method is duration of time. Free-flowing steam is used in two ways.

Continuous Sterilization.—In using this method the steam is applied continuously as long as it is deemed necessary to sterilize the materials. The time is quite variable but should probably be at least one and one-half hours for ordinary apparatus and materials but a longer period for materials which might contain very resistant microorganisms.

Intermittent Sterilization (Fractional Method).—According to this method, sterilization time is broken up into shorter periods since it is believed that sterility is more certain. Each period of heating—from thirty minutes to one hour—is applied on one day. The first day's heating is supposed to destroy the vegetative cells—those in the active growing stage. Between the first and second day's heating, any spores which survived the first day's heating will germinate and develop into vegetative cells. These will be destroyed on the second day. The third period is added to give a greater margin of safety and to destroy any cells which may have survived the first two periods.

The intermittent method was advised at one time for the preservation of fruits and vegetables by canning. The botulism outbreaks, however, from foods treated in this manner, suggested that more reliable sterilization methods should be used.

The significance of boiling in the preparation of foods and in dish-washing is discussed later in this book.

The apparatus used is simple and usually consists of a metallic box with a false bottom up through which the steam will rise. The "steamer" of the kitchen makes a good apparatus although it is small. The supply of steam may come from steam mains or be generated by boiling the water under the sterilizer.

Ironing.—This common everyday procedure in the home is possessed of great hygienic value. Linens and garments are usually thoroughly washed, dried and sprinkled preparatory to the finishing process, ironing. The hot iron generates steam and thus functions as an agent of sterilization. Previous to ironing, the linen may have been washed in boiling water with soap. If the significance of this procedure were realized, it would be looked upon in a different light. Two German investigators studied the effect of ironing on bacteria. They used mechanical ironing machines which reached a temperature of from 100° to 105° C. in

a few seconds, and in a slightly longer time 125° C. The time factor was so measured that every fabric according to the thickness, was exposed to steaming for from twenty-five to thirty seconds. Thick overcoat materials which had been heavily inoculated were found to be freed from staphylococci and *Escherichia coli* (*Bacterium coli*) by the ironing process. The short steam treatment did not destroy spore-bearing bacilli. Large quantities of tubercle bacilli placed in trousers pockets were destroyed by the application of steam for thirty seconds. These investigators were able to sterilize woolen and camel's-hair blankets although the ironing period had to be lengthened to three minutes. When ironing follows thorough washing in hot water and soapsuds, the laundry becomes of added significance in the home. Two German investigators have recommended the use of American steam pressers for disinfection of blankets, infected clothing, etc. It was stated that the temperature between the plates was between 100° and 105° C. The steam presser was said to have many advantages which made it of value in small hospitals.

High-pressure Steam.—It is well known that there is a direct relation between the pressure and the temperature of steam. As the pressure is raised above atmospheric pressure by enclosing it in a confined space, its temperature goes up. The following table gives these relations:

Pressure (Gage)	Temperature	
	Fahrenheit	Centigrade
0	212°	100°
5	228°	109°
10	240°	115.5°
15	251°	121.5°
20	260°	126.5°
40	287°	141.5°

A pressure of 15 lbs. for fifteen minutes has been accepted as satisfactory in some laboratories. However, a pressure of 20 lbs. for from fifteen to thirty minutes gives a greater margin of safety. For materials which may contain very resistant microorganisms, such as soil or other materials which have been in contact with soil, a much longer time must be used.

The apparatus used for sterilizing with high-pressure steam is known under several names, as digester, autoclave, retorts, dressing sterilizer, etc. These are boxes or cylinders made to withstand high pressures. They are fitted with various contrivances to improve their use and admit

of greater convenience. The autoclave is generally the type used in bacteriological laboratories. The dressing sterilizer is an autoclave used in hospitals. It is usually provided with more nickel plate and is a more striking instrument to look at but no more efficient. It may also have an attachment for creating a vacuum in the sterilizing chamber by which dressings are dried before removal from the sterilizer. In canning factories where foods are sterilized in sealed containers by means of heat, the apparatus for sterilizing is spoken of as a retort or cooker.

The source of the steam for high-pressure sterilization may be either a steam main or a generator in close proximity to the autoclave. The former saves much time and is cheaper where steam is generated for other purposes. However, those installations which require the generation of steam close to the autoclave are satisfactory. Such installations are often used in hospitals where there may not be a supply of high-pressure steam from a boiler room.

There is no doubt that many high-pressure steam sterilizers are not giving efficient results. A comparative study of four or five sterilizers two of which were in hospitals, showed that one was not sterilizing and physicians who used dressings and packs sterilized in it were working with false security. Such sterilizers should be frequently tested in order to make certain that materials are being absolutely steril-

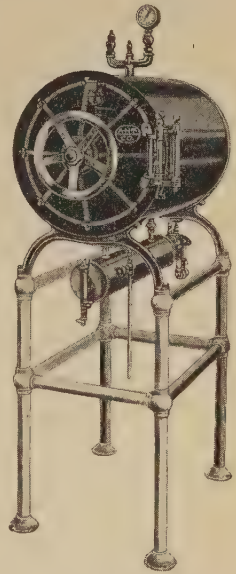


FIG. 93.—High Pressure Steam Sterilizer. (Autoclave.)

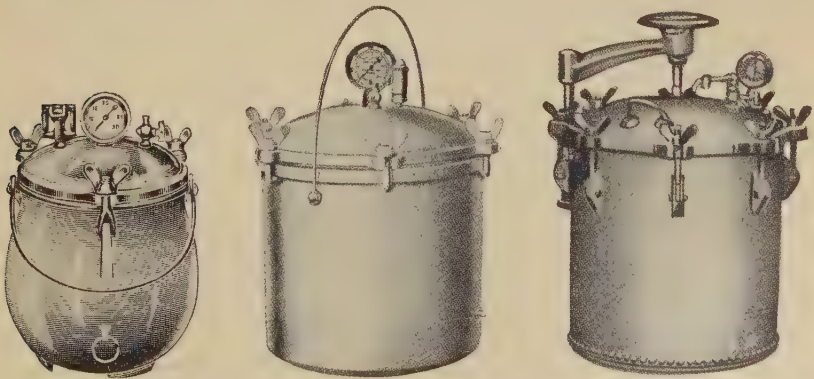


FIG. 94.—Various Types of Pressure Cookers which Are Used for the Sterilization of Canned Foods or Other Materials.

ized. Different methods may be used for checking sterilizers. Mixtures of resistant bacteria may be spread on pieces of cloth in bandages and packings which are then prepared and sterilized in the usual manner. After sterilization, these pieces of cloth may be removed with sterile forceps and placed in flasks of sterile broth for incubation. Another method, involving the use of a little tube containing a material the melting-point of which is known, may be used. If the contents of these tubes are fused, the sterilizer operator knows then that a certain temperature has been reached in the autoclave.

The question is often raised about the efficiency of the smaller types of steam sterilizers known as pressure cookers. They are in reality small autoclaves. One difficulty has been mentioned, however; these sterilizers are frequently provided with only a pressure gage. Both a pressure gage and thermometer should be present in order that one may be certain of the temperature. With a pressure gage and thermometer the operator has two methods of checking the temperature and if he maintains the time, satisfactory results should be secured.

Certain precautions must be kept in mind when using high-pressure steam for sterilization.

1. All air should be expelled from the apparatus. It is apparent that if one has a mixture of air and steam, he will not have the temperature which accompanies the pressure indicated on the gage. The pressure on the gage may be adequate, but if it is due to a mixture of air and steam, the temperature will be too low.

2. There should be a little water to prevent superheating of the steam. This is an almost useless precaution since most steam is too wet and most autoclaves contain more water than operators wish.

3. After the time of sterilization has elapsed, the pressure must be lowered slowly, else the liquid in the containers being sterilized will boil vigorously and may overflow and the cotton plugs in these containers may be blown out.

4. Lastly may be mentioned a precaution upon which there formerly was considerable confusion. It was believed that certain media (food solutions for growing bacteria) containing such substances as lactose, sucrose, etc., would be changed chemically in the autoclave. It was reported that these sugars would be hydrolyzed to the simple sugars; it was suggested that such media should be sterilized by free-flowing steam. Research, however, showed that the latter method caused more hydrolysis than the autoclave sterilization. We now believe that sugars should be sterilized separately as water solution; the necessary amount of this solution may be added to media by means of sterile pipettes.

5. Moist heat is also used at low temperatures. Since a temperature of around 60° C. is used, the time must be greatly lengthened, to six or eight hours for such a medium, as blood serum.

Pasteurization.—This process may be mentioned here although it is not a method of sterilization. It is used for reducing the numbers of bacteria in certain products and, in some cases, may almost completely sterilize them. The application of heat in this connection originated in the early days when practical bacteriology was starting. As before stated, the experiments on spontaneous generation involved the use of heat; the use of too little heat caused erroneous conclusions to be drawn. Pasteur was one of the first to apply heat intelligently for the preservation of such materials as wine, vinegar, and beer. He did not pasteurize milk. Those who have used pasteurization for the purposes just mentioned are between several fires. They must apply the heat for a sufficient time and in sufficient amounts to accomplish the desired results, i.e., the destruction of microorganisms, but, on the other hand, it must not be applied to such an extent that the product is altered.

MOISTURE

Water may also be discussed as a physical agent since it may cause certain changes in the cell mechanically. Why is water necessary for living organisms? All organisms need it to carry foods into the cell and to remove waste products. Such materials must pass through the cell wall. This is especially apparent with single-celled organisms. Water is also necessary to preserve the turgor of the cell. The amount of water for these purposes is probably quite constant although there may be some variation without serious consequences to the cell. With higher forms of life made up of great numbers of cells, the amount required depends on certain external conditions such as evaporation, surface area, etc.

Osmosis and Osmotic Pressure.—When two solutions of different substances or two solutions of the same substance in different concentrations are brought together, diffusion takes place. When these solutions are separated by parchment paper or a similar membrane, it can be seen that a pressure results since liquid will pass through it in either direction depending on the properties of the two solutions. The water molecules diffuse much more rapidly than the solute molecules. The pressure which is produced is spoken of as *osmotic pressure*, and the process which produces it as *osmosis*. Some have suggested that these terms lack clearness and should be dropped from use. Osmotic pressure depends on the number of molecules of solute in solution—the greater the number of molecules the greater the osmotic pressure. Then com-

pounds with large molecules will have a lower osmotic pressure than compounds with small molecules. Salt would therefore exert greater osmotic pressure than cane sugar. Larson, Hartzell, and Diehl reported that a sudden change in the osmotic pressure was detrimental to bacterial cells. From the standpoint of the single cell, three types of solution are possible:

Isotonic solutions, those having a density equal to that of the cell.

Hypertonic solutions, those having a density greater than that of the cell.

Hypotonic solutions, those having a density less than that of the cell.

Isotonic Solutions.—These are solutions of salts having the same density as the cell. The physiological salt solutions used by biologists

are isotonic solutions. Certain writers now speak more specifically of these solutions in terms of the salt used for making them. Thus we may have *physiological sodium chloride solution*, *physiological sodium phosphate solution*, etc. Such solutions are frequently spoken of as normal salt solutions, a designation which is not a good one because the term “normal solution” has a well-defined meaning in chemistry. Physiological sodium chloride solutions are generally 0.85 per cent solutions of salt in water. These solutions are necessary in bacteriology since it is often desirable to preserve bacterial cells intact, as in bacterins. These are agents by which one's resistance to disease is increased since they are to be injected into the blood stream which contains about 0.7 per cent of salt, the use of a physiological sodium chloride solution has another advantage.

Hypertonic Solutions.—Such solutions are more dense than the microbial cell. They therefore contain more salts or other solids.

When two solutions of different densities are separated by a membrane, water passes from the less dense to the more dense. Consequently, when a cell is placed in a more dense solution water will pass out of the cell into the solution and the contents of the cell will become shriveled and granular in nature. The cell loses its turgor. This is spoken of as *plasmolysis*.

The preservation of foods in concentrated solutions is probably due



FIG. 95.—Plasmolysis of Bacteria. (After Alfred Fischer, 1900.)

a, *Vibrio comma* (*Microspira cholerae*). From an Agar Culture in 1.25 per cent Sodium Chloride. They are Plasmolyzed but Still Living; the Protoplasm is Broken Up into Refringent Granules. *b*, The Same Highly Magnified. *c*, *Vibrio comma* (*Microspira cholerae*) Plasmolyzed with Cilium. *d*, Typhoid Bacilli in 2.5 per cent Sodium Chloride Stained; the Cell Contents in Various Positions. *e*, *Spirillum undula* Plasmolyzed Protoplasm is Black in All Cases.

to plasmolysis. Concentrated salt and sugar solutions, and often mixtures of the two, have been used for a long time. Equal weights of sugar and crushed fruit give concentrated solutions which preserve the fruit. Meats may be preserved in strong salt solutions spoken of as brines or pickling solutions. Microorganisms vary greatly in their reaction to concentrated solutions. Some are inhibited at concentrations at which others will carry on an active fermentation.

Plasmolysis has also been of service in studying the structure of bacterial cells. Fischer claimed that the contraction of cell protoplasm away from the cell wall by plasmolysis showed that the protoplasm was not attached to the cell wall.

Hypotonic Solutions.—In such solutions, having a lower density than the cell, water passes from the more dense to the less dense medium. Water, therefore, passes into the cell; the cell swells up and may burst, if the pressure on the cell wall becomes great enough. This is *plasmolysis*. Hypotonic solutions cannot be of great value in practical work since they cannot be secured except under laboratory conditions. Although hypotonic solutions are harmful to bacteria, they cannot be used, for instance, in food preservation. Crushed fruit alone would yield a mixture hypertonic for many microorganisms.

PRESSURE

Bacteria seem to be resistant to high pressures. Fischer reported that putrefaction and fermentation go on under pressures of 300–500 atmospheres¹ and that the spores of *Bacillus anthracis* are unharmed by a pressure of 600 atmospheres for twenty-four hours. The same author called attention to the fact that only a small pressure has to be endured by bacteria on account of their small size. He stated that a coccus 2 microns in diameter at a depth of 7086 meters in the ocean would have to withstand only 90 mg. pressure. Such statements as the above, however, representative of those which appear in many of the older texts, are not in accord with data collected more recently. If sufficiently high pressures are applied for a long enough time, there is a distinct decrease in the number of living cells. High pressures may therefore exert the same effect on suspensions of bacterial cells that freezing does.

We need not be concerned with a presentation of details of experiment nor details of conclusions that have been reached. Larson, Hartzell and Diehl, of the University of Minnesota, found that a direct pres-

¹ An atmosphere is 14.7 lbs. per sq. in. or 1 kilogram pressure per square centimeter.

sure of 6000 atmospheres killed non-spore-bearing bacteria in fourteen hours. A pressure of 12,000 atmospheres for the same length of time was required for killing spores. Having shown that high pressures would destroy bacteria, these authors tried to reach an explanation of the processes involved. They concluded that the factor which destroyed the organisms was the sudden change in the osmotic tension of the fluid in which the bacteria were suspended. Bridgman¹ exposed egg white to a pressure of 5000 atmospheres (75,000 lbs. per sq. in.) for thirty minutes and found that it was slightly stiffened; increasing the pressure to 6000 pounds produced a coagulation like curdled milk, while 7000 pounds caused a complete coagulation. We might suppose that pressure would have the same effect on bacteria.

At the West Virginia Agricultural Experiment Station experiments were conducted to determine whether pressure could be used for the preservation of foods. If pressure could be used, the delicate aromas and flavors of such foods as strawberries, cherries, apple juice, etc., would not be lost as they are by methods involving the use of heat. Hite and his co-workers found that the microorganisms responsible for the spoilage of sweet ripe fruits were largely destroyed. In the case of grape juice a pressure of 100,000 lbs. per sq. in. for ten minutes stopped the fermentation. A pressure of 30,000 lbs. per sq. in. was regarded as the lowest that could be used with significant results. Apple juice exposed to 60,000 and 80,000 lbs. remained sweet without the development of gas.

The effect of a sudden release of pressure on the bacterial cell was studied by Larson and his colleagues. They found that the sudden release in CO₂ pressure caused many of the Gram-negative bacteria to be broken up. The Gram-positive bacteria did not undergo such a destructive change although they were killed. When divers have been under water at great depths for some time, and under the consequent great pressure, they must come up slowly or go through decompression chambers in order not to subject the body cells to the shock of readjustment to atmospheric pressures in too short a time. The same practice is employed with workers in tunnels under rivers where it is necessary to keep the air under pressure to prevent collapse of the walls and roof. Sudden release of high pressures seems to be harmful to some bacteria as well as to man.

Some work has also been done on the influence of gaseous pressure on bacteria. Interest was revived in this subject when it was proposed to freeze ice cream with the air which it ordinarily contains replaced by

¹ Bridgman. Jour. Biol. Chem., 19 (1914), 511 and 512.

carbon dioxide. Two early investigators exposed bacteria in milk to a pressure of carbon dioxide of 52 atmospheres without appreciably harming the bacteria. More recent work also indicates that high pressures of CO_2 will not sterilize liquids. The results with other gases are not widely different from those with carbon dioxide.

AGITATION

The effect of agitation was studied quite early in the days of experimental bacteriology. When investigators were studying the influence of many different factors, the influence of agitation was included. The student approaching this question would anticipate two possible states of knowledge. Agitation would show either a beneficial effect or a detrimental effect. Let us analyze some of the experimental data from the literature on the subject. Horvath in 1878 carried out experiments which have been reported by most of those who have discussed this topic. Gentle shaking was found to be without influence. Vigorous shaking, however, was found to be detrimental. When continued for a sufficient time the bacteria were killed; before that time reproduction was greatly reduced. Meltzer also carried out experiments which indicated that vigorous agitation caused the death of the cells. In most cases those who have reported favorable effect of shaking used very gentle motion while those who report harmful effects have used more vigorous motion. Meltzer also left some cultures of bacteria in an engine house where they were subjected to constant trembling motion. After four days the bacteria in these flasks were dead. From this it has been stated that both trembling motion and very violent motion may be harmful. The matter is quite confused. Undoubtedly much depends on the organism, the type of motion, and other influencing factors.

It is easy to understand how a little shaking might favor cell reproduction. Progeny, as in the case of yeasts, reproduced by budding might be broken from the parent cell and thus more readily make room for more buds. In the case of bacteria reproducing by fission, the process might hasten separation of the two cells, causing them to be in position to divide themselves more readily.

GRAVITY

This physical agent does not have great effect on bacteria. Bacterial cells are mostly water and contain only a small amount of solid matter. Consequently, the specific gravity of the bacteria is not much different from that of the culture media. We have stated before that

our liquid culture media are approximately isotonic solutions. There is a dearth of experimental data on this point. One experiment may be mentioned. Working with an organism called *Bacterium zopfi*, Boyce and Evans were able to influence the type of growth in gelatin stabs by making the growth go upward and by whirling the cultures. In general, the effect of gravity is of negligible significance. With motile organisms the function of movement would, at least, complicate the discussion.

ELECTRICITY

Electricity is a powerful physical agent with which a great number of experiments have been made on bacteria. Some of these have yielded data from which illogical conclusions have been drawn. Experiments were initiated as early as 1875 and consisted in passing the electric current through media in which bacteria were growing or were suspended. Direct currents cause electrolysis with the formation of several different substances such as acids, alkalies, chlorine, etc. These are harmful to bacterial life and have caused the reduction in cells which has been attributed to the electricity. Alternating currents do not cause electrolysis but do cause much heat to be formed. When the temperature is kept low, electricity is said to have almost no effect. To assume that bacterial cells are not directly affected by electricity is about as reasonable as to claim that electricity has no effect on higher animals.

In this connection the author feels that there is considerable misinformation. Certain authors discuss the effect of electricity on bacteria and other microorganisms under two heads, the *direct* effects which are said to be *nil* and the *indirect* effects due to the by-products of the passage of the electric current. This seems to be an unnecessary distinction. The several products of electrolysis are germicidal regardless of their method of preparation. To state that electricity has no direct effects on bacterial protoplasm is not in accord with the facts. No experiment has been carried out in which the electric current has been passed through the bacterial cell. Could such an experiment be conducted there is no reason to suppose that it would not act as it does on other protoplasm. The fact that bacterial cells are colloidal in nature supports the supposition that its colloidal nature would be markedly upset by the electric current.

Attempts have been made to put electricity to practical uses. Among these are attempts to pasteurize milk, but the results have not been entirely conclusive, although considerable data are available to favor the process. The elements of cost enter in all such practices. Attempts were also made to treat sewage. The current was introduced

into the sewage by means of iron electrodes. Improvement was noted, but it was found to be due to the iron salts from the iron electrodes. It was found to be cheaper to purchase the iron salts as such than to make them in the sewage tanks from iron electrodes by means of electric current.

REFERENCES

See the list of reference texts in the appendix.

CHAPTER XI

RELATION OF CHEMICAL AGENTS TO BACTERIA (DISINFECTION)

The relation of chemical agents to the life and development of bacteria is a very interesting subject for discussion; there is, perhaps, no subject in bacteriology and preventive medicine on which there is greater lack of sound information or greater confusion. Many substances have been used for the destruction of undesirable microorganisms; some of these were found to be devoid of activity. The layman who is not trained in the sciences is exploited from all sides with great numbers of different compounds for the prevention of disease dissemination. The use of compounds which are not reliable may lead to the neglect of approved, adequate methods.

Chemotaxis.—Microorganisms show a marked ability to react to the presence of chemical compounds in their environments. All cells exhibit this ability to respond to chemical stimuli. Various kinds of cells differ in this respect and the various chemicals also show a great difference in their influence on cells. The phenomenon of chemotaxis may be defined as the reaction of a microorganism toward chemicals. Some microorganisms seem to be repelled by certain chemical compounds, while others are attracted. Study of this subject requires the use of free swimming cells in order that they may react without hindrance. The phenomenon has been studied with certain salts and with oxygen. It may be profitable to review several of these experiments.

Engelmann studied the subject, using freely moving bacteria that needed oxygen and also bacteria that did not need oxygen. When mixtures of these organisms were placed in water between a cover glass and a slide, those which needed oxygen migrated to the edge of the cover glass where the oxygen supply was most abundant and also where there was opportunity for its regeneration. Those bacteria in the mixture which did not need oxygen and for which oxygen acted as a poison, migrated to the center of the cover glass where they found the conditions of oxygen concentration they preferred. Another unique experiment was carried out to show the same thing. He placed a green algal thread in a drop of water containing these two groups of bacteria and then

directed a small spectrum on it. In those parts of the spectrum where most oxygen was set free, the bacteria requiring oxygen collected. It will be seen in Fig. 96 that this occurred between the lines *B* and *C* and also at *F*. Beijerinck's so-called "respiration figures" are also interesting; they are shown in Fig. 97. The three parts represent horizontal projections of bacteria in drops of water. A small platinum wire was placed at the part represented by the top of the drawing, between the cover glass and slide so as to form a wedge-shaped space, which was occupied by the drop of water, the base of which was in (*m*) the meniscus.

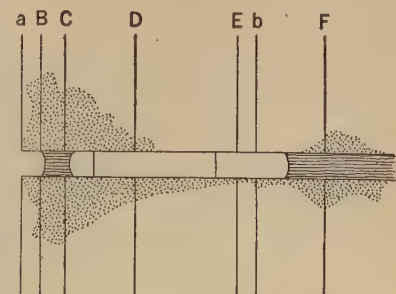


FIG. 96.—Showing the Migration of Freely Moving Bacteria to Areas of Higher Oxygen Concentration about an Alga Thread in a Microspectrum. (After Engelmann.)

The chlorophyll granules in the thread are not shown; the spectrum lines indicate the position of the spectrum.

Pfeffer used still another method of demonstrating chemotaxis. He partly filled a small capillary tube, about 5 to 10 mm. in length and sealed at one end, with the chemical compound to be tested. This tube was

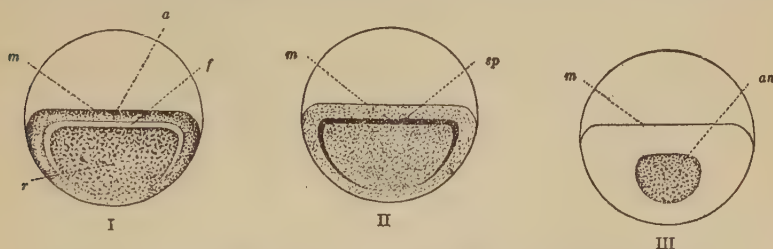


FIG. 97.—Respiration Figures of Motile Bacteria. (After Beijerinck.)

These three illustrations are horizontal projections of bacterial preparations in water. The large circles represent cover glasses. At the top of the circle (cover glass) a small platinum wire (not shown here) is so placed that a wedge-shaped space occupied by the water is formed. The base lies in the meniscus.

I. This respiration figure is of the aerobic type. The motile bacteria collect in the border zone (*a*); the quiescent ones collect at the center (*r*) leaving a vacant space (*f*) between.

II. Respiration figure of the spirillum type. These bacteria need or tolerate but small amounts of oxygen. Therefore, they collect at the circumference of the drop but a little distance in from the edge (*sp*). Here the oxygen concentration is lower.

III. This is a respiration of the anaerobic type. These bacteria migrate to the center of the drop where there is the lowest concentration of oxygen.

then inverted in water made turbid with the bacteria to be studied. A positive chemotaxis was indicated by concentration of bacteria about

the open end of the capillary tube; finally, the bacteria began to enter the tube. Pfeffer studied several common chemical compounds. There seemed to be no relation between disinfecting power and chemotaxis. We might expect that chemicals which acted as disinfectants would repel bacteria, but apparently this is not always the case.

Sterilization and Disinfection.—These are common terms in present-day life. *Sterilization* is the broader and more inclusive term implying

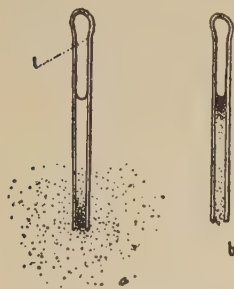


FIG. 97A. — Chemotaxis.
(After Fischer.)

A part of a drop of water containing *Pseudomonas fluorescens* (*Bacillus fluorescens liquefaciens*) and a capillary sealed at one end and partly filled with 5 per cent alkaline peptone solution. Soon after the introduction of the capillary the bacteria have collected about the open end of the tube. This is positive chemotaxis. Later, on account of the need for oxygen, they have collected near the air bubble.

a complete destruction of all living matter in or on a medium or other object. This means the destruction of all living protoplasm irrespective of the kind or type. *Disinfection* is a more restricted term and is applied to processes in which only disease-producing microorganisms are destroyed. The distinction between sterilization and disinfection is often quite artificial since some of our commonest saprophytic, or generally non-disease-producing bacteria, will cause infections under the right set of conditions. Also, it is difficult to select a chemical or other type of disinfecting agent which will kill pathogenic bacteria without having effects on those which are non-pathogenic. Generally speaking, disinfection may be sterilization; certainly sterilization is disinfection. Agents and procedures that destroy such organisms as bacteria, germs, algae, protozoa are spoken of as *bactericides*, *germicides*, *algicides*, and *protozoacides*, respectively. An antiseptic or preservative is an agent or process which inhibits microorganisms but does not necessarily kill

them. Quite often an antiseptic will destroy some special function of the organism such as ability to reproduce or grow, form pigments, ferment, etc. It is not easy to distinguish between some of these terms. It will be seen that the definitions of an antiseptic and disinfectant distinguish these agents. In practical disinfection, however, they are used in such manner by some individuals as to confuse the whole field of disinfection. The difficulty may rest on the fact that there are no methods for separating disinfecting ability from the antiseptic ability. A substance, therefore, may be a relatively good antiseptic but a poor germicide or disinfectant. The distinction must be made between these two functions. The time factor may enter into the discussion. An antiseptic might produce the same results as a disinfectant but it would have to be

applied for a long time. Such distinctions should be kept in mind when reading the directions on labels such as those which appear on mouth washes, gargles, sprays, etc.

Characteristics of a Good Disinfectant.—An ideal disinfectant probably does not exist. However, it must possess certain characteristics each of which may now be taken up. They were listed by Dreyfus in the American Journal of Public Health (1912) as follows:

1. *High Germicidal Power.*—Unless a chemical has an appreciable germicidal power, it does not come within the scope of this discussion; consequently, it is reasonable to present this as the first requirement of a good disinfectant. A chemical may be a good disinfectant for one bacterium but be inactive toward another. This is one reason why it is impossible to make general statements about the general utility of disinfectants. Disinfectants show, in many cases, a marked specificity for certain bacteria.

2. *Stability.*—This requirement demands that the disinfectant be fairly stable and not have a marked affinity for organic matter. If it does react readily with organic matter, it will be used up quickly and not be available for action on the bacteria. With such disinfectants a great excess would have to be used to satisfy the demands of the organic matter and leave enough to function as a bactericide. It is easily understood that a compound, which in larger amounts might be an active disinfectant, might be so reduced that it would become merely a preservative or antiseptic. A good disinfectant should also be stable and not undergo spontaneous decomposition. This is exemplified in hydrogen peroxide, which often loses its oxygen and thereby becomes inactive.

3. *Homogeneous Composition.*—This is one of the most significant requirements of a good disinfectant. Certain compounds would be good disinfectants if it were not for the fact that they form emulsions in water rather than homogeneous solutions. The cresols, for instance, are powerful disinfectants, but form milky emulsions in water; consequently, as we will see later, they have been treated especially to make them homogeneous. They have been saponified to yield states which are soluble in water.

4. *Ready Solubility in the Strength Required for Disinfection.*—Before a chemical can function as a disinfectant, it must, of course, be soluble in some material such as water, alcohol, etc. It is not necessary that it be extremely soluble, but only in concentrations in which it will function as an active disinfectant. We will find later that mercuric chloride is, under certain conditions, an active disinfectant. Mercurous chloride, however, cannot be used as a disinfectant on account of its insolubility in water. This requirement is closely connected with the ionization of chemicals in water solution. In many cases disinfection is best explained on the basis of the ions which are formed in a water solution.

5. *Non-poisonous to Animal Life.*—The ideal of disinfectants is one which is not poisonous to the higher animals in the concentrations which are used for

disinfection. Of course, the chemical compound would have to be poisonous to protoplasm, at least certain types of protoplasm, or else it would not act as a disinfectant. It is unreasonable to assume the existence of a chemical compound, which is non-poisonous to the protoplasm in higher animals and extremely poisonous to the protoplasm in bacterial cells. It is difficult to select a compound which will not harm the tissue but which will destroy the parasite. It is well illustrated with the group of disinfectants that have been tried out for such infection as sore throats. Many of these are worthless in the concentrations used; they could not be used in higher concentrations on account of the fact that they would attack the tissue in the throat and thus make it more susceptible to infection.

6. *Non-Corrosive*.—Corrosive chemical compounds are very difficult to store, and their strength is often greatly reduced by their action on the container. Quite often the compound is reduced in strength in order to retain as many of its disinfecting properties as possible, and to avoid its corrosive action.

7. *Sufficient Power of Penetration*.—Disinfectants are often needed for the deeper layers of tissue, and it is obvious that their application to the surface might not result in benefit. Few compounds have good penetrating powers, and physicians and surgeons find it necessary to apply the disinfectant in the immediate vicinity where it is needed. To illustrate, we have had numerous pharmaceutical products prepared for the treatment of pyorrhea. This infection is a deeply seated abscess at the base of the teeth, and it is silly to expect that a disinfectant incorporated in a dentifrice would destroy the bacteria in such abscesses.

8. *Moderate in Cost*.—This prerequisite cannot be considered by itself. It must be considered along with the germicidal power of the compound. However, it is usually the second prerequisite that enters into the selection of the disinfectant. First, one wishes an active chemical, and secondly, one that is not too expensive. The selection of a disinfectant with regard to cost is often determined by the amount that will have to be used.

Dreyfus added the following two characteristics but it is doubtful whether they should be required of a disinfectant. They add properties to a compound which are set apart from the disinfecting property. They are included here only for completeness.

9. *Deodorizing Properties*.—Few disinfectants are deodorants. It would be a fine combination of characteristics if disinfectants had deodorizing properties. However, they merely replace one odor with another, and some of our most useful disinfectants, such as the phenolic group, possess odors which are disagreeable for some people.

10. *Power to Remove Dirt and Grease*.—This is also an unnecessary demand on a disinfectant. If a detergent is needed it is better to purchase one separately and to follow its use with a good disinfectant. It is extremely difficult to find a compound which will fulfill the eight requirements of a good disin-

fectant and also the ninth and tenth. This is well emphasized by the low disinfecting ability of soap, even the so-called germicidal soaps.

Factors Influencing Disinfection.—First of all, it should be stated that disinfection is in all probability a chemical reaction. At least much good will result from considering it from this viewpoint. Two chemicals are reacting, the disinfectant and the bacterial cell, or to express it in another way, the bacterial molecules and the chemical molecules. What factors influence chemical reactions and how may they influence the chemical reaction between the molecules of disinfectant and bacteria?

1. *Temperature.*—All chemical reactions are greatly speeded up by raising the temperature. The chemist, by raising the temperature, is able to accomplish in the laboratory in a few minutes what would require a great number of years to accomplish at room temperature. In this sense heat may be looked upon as a catalyst, since it drives reactions very rapidly which are taking place very slowly at ordinary temperatures. It has been found to have the same effect in disinfection. With *Salmonella paratyphi* (*Bacterium paratyphosum* A) the killing power of carbolic acid has been found to be increased from two to four times for each 10° rise in temperature. This increase in the disinfecting activity of a compound by raising the temperature is spoken of as its temperature coefficient. We will learn later that disinfection is probably a coagulation of protein. The effect of heat in the coagulation of proteins is nicely illustrated in the boiling of an egg. The protein could be coagulated (boiled) at 90° C. in perhaps two or three hours, but by raising the temperature to 100° C. The coagulation time is greatly shortened to a few minutes. At temperatures below 90° C., the time would be proportionally longer.

2. *Time.*—It is impossible to separate temperature and time, but considerable good will be derived if we attempt to separate them for this discussion. We have just learned that by raising the temperature we may greatly shorten the time required for disinfection. One of the important practical facts about disinfection is that it is a time process and not an instantaneous reaction. Consequently, in reading data about disinfection and the activities of certain compounds, the information is much more specific if the time is given. For instance, it does not mean much to say that an organism is killed at 80° C. It means much more to say that this same organism is killed at 80° C. in five minutes.

3. *Moisture.*—It has been stated above in several places that disinfection is probably a coagulation of protein. It has been well demonstrated by biochemists that the coagulation of protein requires water. It is also possible that water is necessary for carrying the heat and chemicals, if they are being used, into the cell. In this way water may serve a two-fold purpose in disinfection. In the chemical laboratory the compounds are usually handled as water solutions since their reactivity is markedly increased in the liquid condition. Chick and Martin have stated that proteins are coagulated in two steps: first, there is a reaction between water and the protein, a process

referred to as denaturation; secondly, there is an agglutination or separation of the altered protein. The necessity of water in disinfection was nicely shown by one investigator with chlorine. He exposed dry anthrax spores in an atmosphere containing 44.7 per cent of chlorine for one hour without killing them. When they were moistened they were killed in this time by only 4 per cent of chlorine. It is also stated that absolute alcohol is not a disinfectant but shows some disinfecting ability when used in 50 per cent solution.

4. *Concentration of Reacting Substances.*—In disinfection reactions, the reacting agents may be regarded as chemical molecules and bacterial molecules. From our knowledge of chemistry it will be seen that, within certain limits, the more concentrated these reacting substances, the more product will be formed. The product in this case is killed bacterial protoplasm. While there are important objections, disinfection may be looked at from the standpoint of the mass law. The mass law states that the amount of reaction depends on the concentration of reacting substances. Consequently in disinfection the greater number of cells (bacterial molecules) and the more chemical molecules there are present, the greater should be the amount of product or destruction of bacterial cells.

Disinfection is a chemical process and therefore probably follows chemical laws. Chick (1908), among many others, has given considerable study to this subject and many of her data form the basis for our knowledge on disinfection. The chemical reaction in the present discussion would take place between the disinfectant and the bacterial protoplasm. Chick stated that disinfection followed the well-known law of physical chemistry—the monomolecular law.¹ The same equation may be used provided legitimate substitutions are made in it to make it fit disinfection.

$$\frac{-dc}{dt} = KC$$

or

$$\frac{1}{t_2 - t_1} \log \frac{c_1}{c_2} = K.$$

This may be changed to

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K.$$

In this equation c_1 and c_2 have been substituted for n_1 and n_2 , the numbers of surviving bacteria. For such a discussion, we may define the monomolecular law as one governing a reaction between two substances where the change in concentration of one may be measured. This law seems to be followed in

¹ Students of disinfection are not agreed that this law should be used for explaining it. In an introductory book of this nature it is unnecessary to present all of the various opinions. This discussion is presented to stimulate those students who have the necessary background to think over the disinfection process. The various arguments that have centered about the explanation of the process may be left for future study.

many cases of disinfection when any agent is used to destroy bacteria. It is apparent that the rate of death is always proportional to the number of cells which are living at any given time. This simply means that where there are larger amounts of reacting substances, more reaction is secured. Consequently a greater drop is secured in the curve during the first units of time. Some objections may be raised against regarding disinfection in this light. The bacterial cell must be regarded as a molecule. It is quite different since it possesses "life" and is surrounded by a membrane. It is also necessary to neglect the change in concentration of the disinfectant. At present the disinfectant is usually added in such excess that any change which might occur, is neglected.

5. *Presence of Extraneous Matter.*—The chemist tries to experiment with very pure reagents. He does this in order to be able to secure more trustworthy data, and also to allow the reactions to go on more rapidly. We must keep the same ideas in mind in disinfection. The presence of great amounts of organic matter, other than that in the bacterial cells, greatly reduces the effect of disinfectants. In such cases it is necessary to use amounts of disinfectants far in excess of those required when organic matter is almost absent.

6. *Surface Tension.*—This is one of the newer factors that has been found to influence disinfection. Larson and Montank found that by lowering the surface tension of the medium *Mycobacterium tuberculosis* became so altered in characteristics that it was more susceptible to the action of immune bodies in animals. Investigations in other laboratories have confirmed this observation that surface tension plays an important rôle in disinfection. Frobisher, for instance, stated that the bactericidal powers of phenol and hexylresorcinol were increased by lowering the surface tension.

Specificity of Disinfectants.—Some disinfectants have a special action on certain bacteria; consequently general statements about the disinfecting properties of compounds are without significance. Chlorine compounds, for instance, are known to be almost devoid of action, in ordinary concentrations, on the tuberculosis bacteria but quite active on other bacteria. Some students of the disinfection problem have suggested that each chemical compound proposed for use as a disinfectant should be studied with the micro-organism for the destruction of which it is proposed by the manufacturers. This would allow the public to work with more certainty in its selection of disinfectants.

Action of Disinfectants.—Just how are bacteria killed by the various disinfectants? The several factors which influence disinfection have just been discussed from the viewpoint that disinfection is a chemical reaction following well-established laws. It may be helpful to retain this chemical viewpoint in this paragraph also in trying to determine how the bacterial cells are killed. For convenience the following reactions may be suggested:

I. Oxidation Reactions.

- a. Chlorine and other compounds.
- b. Hydrogen peroxide.
- c. Ozone.
- d. Potassium permanganate.

II. Hydrolyses.

- a. Strong acids.
- b. Hot water.

III. Formation of salts with proteins.

- a. Salts of the heavy metals.
- b. Direct halogenation.

IV. Coagulation of the proteins in the cells.

This tabular outline may cover the several processes which are involved in the destruction of bacteria. It is not easy to prevent overlapping. Protein coagulation, although singled out as a separate process, may be the explanation of the action of all of the other reactions. Even though a cell is killed by an oxidizing disinfectant, it may be finally destroyed by the coagulation of the proteins. It is quite possible that we do not know just how disinfection takes place and what internal mechanism of the cell is destroyed.

We may now take up consideration of the larger groups of chemical compounds which have been used as disinfectants. These compounds may be classified in different ways. Any method of classification or grouping would be arbitrary. Consequently, in this chapter the compounds have been grouped after a scheme used by Dakin and Dunham in their "Handbook of Antiseptics." Lack of space will prevent an exhaustive discussion and consequently only some of the more commonly used compounds will be mentioned.

I. THE HALOGEN COMPOUNDS

The halogen compounds, chlorine, bromine, fluorine, and iodine are very active chemically, so active that they do not exist in the free state. They have a marked affinity for living proteins—a fact which every student in chemistry soon learns from experience. This affinity for living protoplasm gives some of the halogen compounds a place among disinfectants. Not all of the members of this group may be used.

Chlorine Compounds.—Several of the chlorine compounds have played important rôles in preventive medicine. They have been used for disinfecting public water supplies, for the treatment and disinfection

own
new
killed
bacteria

De Fine
sterilization
disinfection
antiseptic

tion of war wounds, etc. The disinfecting action of these compounds may be explained in two ways, *oxidation* and *chlorination*. The chlorination explanation for the activity of chlorine compounds as disinfectants seem reasonable but the oxidation explanation less so. However, these compounds disinfect by oxidation in the same manner that they bleach fabrics by oxidation. The chlorine replaces oxygen in water, liberating nascent oxygen which attacks the bacterial cell. The chlorine also disinfects by direct union or by chlorination. According to this explanation the chlorine atoms add themselves directly to the protein molecule or replace constituent atoms such as the hydrogen atoms in $-\text{NH}_2$ groups in the amino acids. Protoplasm which has been chlorinated in this manner cannot function normally.

Not all chlorine compounds act as disinfectants. In this connection the chlorine compounds may be divided into two groups. One group contains those compounds which have the chlorine firmly bound as it is in sodium chloride. The other group has its chlorine loosely bound as in calcium hypochlorite. As has been mentioned in other places, this may be a disadvantage for the compound may deteriorate during storage and later be used with a false sense of security.

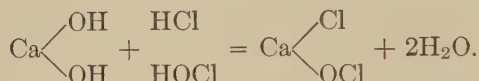
Liquid Chlorine.—This is sold in cylinders and has its greatest use in the disinfection of water where it has almost entirely replaced calcium hypochlorite. Liquid chlorine has the advantage over calcium hypochlorite that only active chemical is added to the water. When calcium hypochlorite is added considerable inactive matter is included. The action of chlorine on living protoplasm is well known to students of chemistry. It is very irritating when it is inhaled in large amounts.

Chlorine in Treatment of Respiratory Diseases.—The treatment of respiratory infections with chlorine received considerable discussion several years ago. One investigator reported that beneficial results followed the administration of chlorine gas to individuals suffering from rhinitis. The original investigators reported that inhalation of 0.015 mg. of chlorine per liter for one or more hours had a distinctly curative value in common respiratory infections such as colds, or whooping cough. Experimental work to verify these statements was carried out in the laboratories of the New York City Department of Health. The results were quite different from those reported by the originators of the method. Several other investigations were reported all of which refuted the claim that chlorine gas, in the concentrations mentioned above, had a germicidal effect on mucous membranes and cured respiratory infections.

Calcium Hypochlorite.—This is also known as chloride of lime, hypo, bleaching powder or "bleach." This compound has had great use in sanitation and has probably been responsible for great saving of life

from water-borne diseases. It is a relatively cheap compound but has the disadvantage that it deteriorates easily, and unless one satisfies himself of the strength of the lot which he is using he may use it with a false sense of security. This compound is prepared by passing chlorine over lime. The chlorine is not very firmly bound as it is in sodium chloride (NaCl).

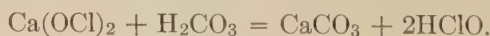
Chloride of lime is soluble according to Hooker (1913) in about twenty times its own weight of water. It leaves an insoluble residue composed mostly of calcium hydroxide. It is prepared from Ca(OH)_2 as follows:



The HOCl is made by treating water with chlorine. Some care should be used to secure clear solutions for use in disinfection. This is especially true in water treatment. The potential part of this compound is very soluble in water while the part with no sterilizing properties quickly settles out. Hooker (1913) gave the following rules for the use of chloride of lime:

First, do not mix too stiff a paste, otherwise a gelatinizing action takes place and greater difficulty in settling out is encountered. Never mix a paste with less than $\frac{1}{2}$ gal. of water for 1 lb. of chloride of lime. Second, it is not necessary nor desirable to grind nor break up the lumps too thoroughly; the available chlorine nearly all dissolves readily and too much agitation is detrimental to prompt settling.

The above precautions should be borne in mind in any application of chloride of lime to sanitation. The strength of chloride of lime is measured in terms of "available chlorine." This is an unfortunate term since it implies that the disinfecting properties of the compound rest solely in the chlorine. Past work indicates that it is an oxidation as well as a chlorination. The oxygen is formed in the following manner:



It has been suggested by some sanitarians that the term "available chlorine" be replaced by the term "potential oxygen." Lager (1916) made some interesting statements in regard to the action of calcium hypochlorite. He stated that the disinfecting action does not depend upon the available chlorine or the length of time the chlorine acts. He states that the time for killing depends upon the resistance of the organism.

The United States Pharmacopoeia specifies that chloride of lime shall contain 35 per cent of available chlorine. As it is purchased in small cans on the open market it may fall far short of this and the use of such a product allows a false sense of security. Hooker, in the book mentioned above, presents a table which contains much useful information. It appears here as Table IV and is based on 30 per cent bleach.

TABLE IV
SHOWING STRENGTH OF SOLUTIONS OF CALCIUM HYPOCHLORITE
(After Hooker, 1913)

Pounds Bleach per 1,000,000 Gallons Water	Parts Bleach per 1,000,000 Parts Water	Parts Chlorine per 1,000,000 Parts Water	Grains Bleach per Gallon Water	Grains Available Chlorine per Gallon Water	Drops Bleach Solution, 2 Per Cent or $\frac{1}{2}$ Lb. in Gallon Used per Gallon Water
2	.24	.08	.104	.005	.25
4	.48	.16	.028	.009	.25
6	.72	.24	.042	.014	.75
8	.96	.32	.056	.019	1.00
10	1.20	.40	.070	.023	1.25
12	1.44	.48	.084	.028	1.50
14	1.68	.56	.098	.033	1.75
16	1.92	.64	.112	.037	2.00
18	2.16	.72	.126	.042	2.25
20	2.40	.80	.140	.047	2.50
22	2.64	.88	.154	.051	2.75
24	2.88	.96	.168	.056	3.00
26	3.12	1.04	.182	.061	3.25
28	3.36	1.12	.106	.065	3.50
30	3.60	1.20	.210	.070	3.75

A few objections have been made against chloride of lime. It takes the color out of fabrics, etc., and exerts great reactivity with organic matter. When it is used in the presence of large amounts of organic matter, a sufficient amount must be used to satisfy the requirements of that matter and still leave enough left over for destroying the bacteria. This is difficult to determine.

Labarraque's Solution.—This is a solution of chloride of lime containing 2.4 per cent of available chlorine.

Dakin-Carrel Solution.—This solution has been found to be of great value in the treatment of wounds. Different methods may be used in the preparation of this solution. Below are two formulas:

Formula No. 1.—Dissolve 140 gm. of sodium carbonate in 10 liters of distilled water and add 200 gm. of calcium hypochlorite. Shake this solution thoroughly and allow the sediment to settle. After sedimentation has taken place, decant the clear liquid and add 40 gm. of boric acid. The solution is then ready to use.

Formula No. 2.—Dissolve 100 gm. of sodium carbonate, 80 gm. of calcium hypochlorite in 10 liters of water. This should give a solution of about $\frac{1}{2}$ per cent of hypochlorite.

In the use of this solution the wound should be kept constantly moist. Doctor Carrell applied the solution every hour or two hours, the amount used generally depending upon the character of the wound. Generally 5 to 10 cc. were applied every two hours. The use of this solution was also extended to other infections such as puerperal sepsis, etc.

The absolute germicidal power of the chemical was studied by Nissen (1890). He used pure cultures and secured the following results which are quoted from Hooker:

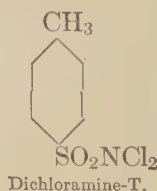
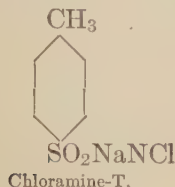
Organisms* Killed	Minutes	By Dilutions of Chloride of Lime
Bacillus typhosus.....	1	1-800
Bacillus typhosus.....	10	1-1600
Bacillus cholerae.....	1	1-800
Bacillus cholerae.....	10	1-600
Bacillus anthracis.....	1	1-1000
Staph. pyogenes aureus.....	5	1-800
Streptococcus erysipelatus.....	1	1-200
Anthrax spores.....	15	1-20
Anthrax spores.....	20	1-100

* The older names for these microorganisms have been used in this table. The newer ones are given at different places in this book.

Hooker has collected an excellent résumé of the literature up to 1913.

Other commercial disinfectants have been built up with calcium or sodium hypochlorite as the bases. Some of these are antiformin, eupad, eusol, etc. These need not be discussed in detail since they act in the same manner as those compounds which have been discussed.

Chloramine-T and *Dichloramine-T*:



Toluene parasulfondichloramin is formed when nascent chlorine, formed in aqueous solutions of hypochlorite, is brought in contact with suppurating wounds. Dakin (1916, 1917) has proposed the name dichloramine-T for this compound. He has shown that most of the substances containing a NCl group are strongly germicidal. The presence of more than one such group does not seem to increase the germicidal properties to any marked degree. It may be used in stronger concentrations than some of the other disinfectants. It is claimed that its use is as simple as the use of tincture of iodine.

Iodine Compounds.—These react in about the same manner as the chlorine compounds.

Iodine.—This is regarded by many as a reliable, efficient disinfectant used usually as a "tincture" which is a solution in alcohol of varying strength. A 2.5 per cent solution in 70 per cent alcohol was advised by Dakin and Dunham. Iodine has been used for the disinfection of wounds and skin. It seems from recent work that it has enjoyed a false reputation as a surface sterilization agent. Iodine is quite irritating in certain parts of the body and does not satisfy the requirements that a disinfectant should be efficient and still non-injurious to delicate tissues of the body. The doubtful value of iodine under some conditions is borne out by serious infections following its use. It should also be borne in mind that there is specificity among disinfectants. Some disinfectants are more active against one organism and less active against others. Mahon and White (1915) report that iodine in alcohol is about four times as powerful as a disinfectant as phenol. Their work was done on naked organisms with *Eberthella typhi* (*Bacterium typhosum*) as the test organism.

Iodoform.—This compound has antiseptic properties. It is believed to break up slowly in the presence of organic matter, forming free iodine. The free iodine is said to disinfect. Eugling (1912), in studying the germicidal action of iodoform, could not detect a reducing action of bacteria resulting in the liberation of free iodine.

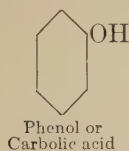
Bromine Compounds.—These are not used as disinfectants on account of their causticity. Bromine is used externally in 3 per cent solution. Since it is a strong oxidizing agent, it attacks metals readily. Its properties place marked limitations on its use as a disinfectant.

II. PHENOLIC GROUP OF COMPOUNDS

No compounds are more useful than these when all of the characteristics of a good disinfectant are considered. They are relatively cheap and very active. However, many of them possess pronounced odors which are objectionable to some persons. The phenolic group of

disinfectants includes the so-called "coal-tar" compounds. These are prepared by the destructive distillation of coal and wood.

Phenol (Carbolic Acid).—As was stated in the chapter on the History of Bacteriology, phenol or carbolic acid was permanently established in surgery by Lister. Phenol is usually used in a 5 per cent solution although it may be diluted a little more if a 5 per cent solution is too strong. Pure carbolic acid is solid at ordinary temperatures. On account of this, pharmacists dilute it (9 parts of pure phenol to 1 part of water) to make Phenol Liquefactum, U. S. P. Dorset recorded the advantages and disadvantages as follows:



The advantages are:

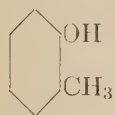
- (1) It is reasonably effective for destroying non-spore-bearing bacteria.
- (2) Its action is only slightly interfered with by albuminous substances.
- (3) It does not destroy metals or fabrics in a 5 per cent solution.
- (4) It is readily available at all pharmacies.

The following disadvantages may be mentioned:

- (1) It cannot be depended upon to destroy the spores of such bacteria as anthrax and malignant edema.
- (2) It is expensive (the pure phenol costs approximately 75 cents per pound).
- (3) The odor is offensive to some people.

Another form of carbolic acid is also available under the name "crude carbolic acid."

The Cresols (Methyl-phenols).—The cresols are methylphenols. Three are possible, depending on the location of the —CH_3 and —OH groups in the molecule. The cresol on the market is a mixture of these three isomers. Many of these contain impurities which do not greatly impair their disinfecting properties. Most of the commercial cresols are guaranteed as to strength. For general disinfection a 2 per cent solution is sufficient. Cresols are not soluble in water, and this has caused the preparation of several modifications.



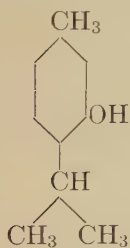
Lysol.—In order to avoid the insolubility of the cresols and the formation of a milky emulsion, they have been saponified to make a soluble compound. The best known is lysol, which is prepared by saponifying crude cresols with linseed oil in the presence of alcohol. This gives a mixture with a soapy appearance. Lysol is efficient in 2 per cent solution and should be in every home. It is, perhaps, the most valuable

disinfectant which we have. *Liquor cresolis compositus*, U. S. P., and creolin are other cresol disinfectants like lysol.

The advantages of lysol as stated by Dorset are:

1. A 2 per cent solution of cresol is as efficient as a 5 per cent solution of carbolic acid.
2. It is not interfered with by albuminous substances.
3. It is cheaper than carbolic acid.
4. It does not destroy metals or fabric in a 2 per cent solution.
5. It is more effective than carbolic acid for destroying spores of bacteria, such as those of *Bacillus anthracis*.

The action of the cresols as disinfectants has received study by Cooper (1912). He found that the introduction of the methyl (as well as the nitro) group increased the bactericidal and protein penetrating powers of phenol while the entrance of the hydroxyl group decreased these properties. Solutions of phenol in alcohol and in fat had no bactericidal action on spores and did not penetrate solutions of gelatin. The precipitating action of phenol was increased by adding acids. Cooper pointed out that the selective action of phenol on different bacteria is connected with different susceptibilities of proteins to its precipitating action. The adsorption of phenol by bacteria is only the beginning of disinfection. The real explanations for the action of the cresols need not be reviewed in a book of this nature. The cresols on the market are mixtures of the three, ortho-, meta- and paracresols. If the disinfecting value of the three cresols varies, as was reported by Cooper, the disinfecting value of any mixture would depend on the concentration of the three components.



Thymol.—This is propylmethylphenol; although it appears in many compounded products which are supposed to possess germicidal properties, it is exceeding weak in its action on bacteria. It is especially common in dentifrice preparations. We may leave it as scarcely worthy of the name of antiseptic.

III. SALTS OF THE HEAVY METALS

The soluble salts of some of the heavy metals exert a destructive action on bacterial protoplasm. In water solutions they ionize and the metallic ion reacts with the protoplasm of the cell. In this manner salts are formed with the metallic ion and the proteins. These are spoken of as mercury-proteinates, silver-proteinates, copper-proteinates,

etc. The value of the metallic salts is connected, then, with their solubility in water at least in the concentration necessary for disinfection.

Lusini (1912) pointed out with regard to the cations that, although the disinfecting property is not a function of the atomic weight, the heavy metal exerts the greatest action. With a few exceptions, the disinfecting action increases with the chemical affinity of the combined elements. The alkali group is less active than the alkaline earth group. The last-mentioned group does not agree with the generalizations mentioned above since its disinfecting power is in inverse ratio to the atomic weight. Among the other elements of the periodic system iron has a lower activity corresponding to its lower atomic weight; tin has a higher and lead still higher activity. This has been confirmed, in general, by other investigators. Bitter (1912) found that the following metals exerted an antagonistic action in the order given: copper, brass, silver, gold, platinum, lead, cast iron, steel, aluminum, nickel, zinc, and tin. The action was found to be the same for both corroded and polished metal. Such data are interesting when it is recalled that some of these metals are used for mouthpieces on drinking fountains, etc. Under such conditions, however, it is very doubtful whether germicidal properties would be shown.

Mercuric Salts.—*Mercuric chloride*: Mercuric chloride (HgCl_2) has long been used in disinfection. It does not fulfill all the requirements of a good disinfectant but does fulfill several of the more important ones. It is very poisonous to higher animal life and reacts readily with metals used in containers. The toxicity for higher animals requires that it be used in very dilute solutions in disinfection. Its affinity for proteins has caused its introduction for other purposes. It is used for the preservation of zoological specimens. Cases of poisoning in human beings are treated with egg white on account of the affinity which mercuric chloride shows for this material. Solutions of different strengths are used in disinfection work. A solution of 1 to 1000 for use in the presence of organic matter is sufficient. Mercuric chloride is soluble in sixteen parts of water.

Mercurochrome-220.—This is a complex chemical compound known as dibromooxymercureylfluorescein which has been introduced into preventive medicine as a disinfectant. It has been especially proposed as a skin disinfectant with which to replace iodine. It has characteristics which make it more desirable in that it is not as caustic and its use, therefore, is not attended with as much pain. It has also been used as an internal disinfectant in urinary and systemic infections. It seems to have a marked action on the pyogenic microorganisms. It has one disadvantage of possessing a deep red color which stains the skin. The

manufacturers suggest this as an advantage since one can tell more quickly the extent of the surface which has been disinfected.

Zinc Salts.—The zinc salts are feeble disinfectants. They are mentioned here only to emphasize that fact. Some of them like zinc chloride appear in widely advertised dentrifices the activity of which as disinfectants is explained on the basis of the zinc chloride content. Some of the statements on the literature accompanying these preparations are very misleading and may result in the neglect of approved methods. Laboratory experiments have, in some cases, shown that such preparations are devoid of action on bacteria. McClintic (1905) found that *Escherichia coli* (*Bacterium coli*) was not killed in one hour's exposure to a 5 per cent solution of zinc chloride and that it required ten minutes for a 25 per cent solution to kill the same microorganism. The spores of *Bacillus subtilis* and *Bacillus anthracis* were found to resist 100 per cent and 50 per cent solutions thirty and forty days respectively.¹

Silver Salts.—Like other inorganic salts silver precipitates proteins from solutions as insoluble silver proteinates. It probably acts in the same manner on bacterial protoplasm. Biassoti (1910) reported that colloidal silver electrically prepared would in a few hours kill *Staphylococcus aureus*, *Eberthella typhi* (*Bacterium typhosum*) and *Corynebacterium diphtheriae* (*Bacterium diphtheriae*). The silver is probably united to the protoplasm in some manner since Gram-positive bacteria are made Gram-negative. Simpson and Hewlett (1914) experimented with colloidal silver in the form of "collosols" and found them to be active disinfectants against *Eberthella typhi* (*Bacterium typhosum*). The collosols were said to be non-poisonous, slow of action and very expensive. Silver is also combined with a number of other materials which are used as disinfectants. *Argyrol*, a silver salt of vitellin, is used in concentrations of 1 to 4 per 1000 for disinfection of some of the mucous membranes. *Protargol* is another silver-protein compound. *Albargin* is a silver salt of galactose. It is used in treatment of certain of the venereal diseases. It contains from 10 to 15 per cent of silver. *Argentamin*, a 10 per cent solution of silver nitrate in 10 per cent ethylene-diamin, has been used for the treatment of gonorrheal infections. It is usually diluted to about 1 part in 300 parts of water. *Argentose* is a silver compound of nucleoprotein and is used much as silver nitrate is used. *Argonin*, a silver caseinate, is used in 3 per cent solution for the treatment of gonorrhea and ophthalmia neonatorum (eye infections in infants newly born).

Silver Nitrate, AgNO₃.—This silver salt has marked affinity for proteins as indicated by the darkening effect on the skin. It is not quite

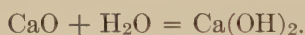
¹ See Journal of the American Medical Association, Vol. 73 (1919), 1380-1381.

as active as mercuric chloride but does not react as readily with extraneous proteins as does the latter. It is quite caustic and in solution of 0.1 to 1.0 per cent has been used as an eye disinfectant to prevent congenital blindness in infants.

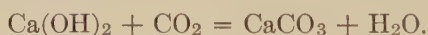
Copper Salts.—The copper salts are used as much to-day as in former times. They function to-day as algicides for the treatment of public water supplies. About 10 lbs. of copper sulfate per million gallons are required to rid a reservoir of objectionable algae which, during the late summer, may impart bad odors and tastes to the water. As a disinfectant copper sulfate has been found to possess some value. Since other disinfectants are now available, little more need be said beyond the statement that copper salts, in general, are not active.

Calcium Salts.—With the exception of calcium hypochlorite which has been discussed before, and lime to be discussed now, the calcium salts do not hold an important place among disinfectants.

Calcium Oxide.—This is known as quick lime and is very caustic in the presence of organic matter. It may be used in two forms, as slaked lime or milk of lime. Slaked lime is prepared by adding a pint or pound of water to 2 lbs. of lime. The reaction proceeds as follows:



This will react with carbon dioxide to form calcium carbonate according to the following equation:



Milk of lime is a thick mixture containing four to six parts of slaked lime. This milk of lime reacts with carbon dioxide to form the carbonate as does slaked lime. Therefore, these mixtures should be used as soon after preparation as possible.

IV. MISCELLANEOUS COMPOUNDS

A few miscellaneous compounds which are used in practical disinfection may now be discussed. Most of those which will be discussed here are used by the layman in everyday life.

Hydrogen Peroxide.—This is an oxidizing disinfectant which is probably far less valuable than many think. It must be used in excessive amounts if it is to be active. It is probably too reactive to be a safe reliable bactericide. It is quickly broken up in the presence of organic matter and the oxygen dissipated. It is possible that as a surface disinfectant it might have some value but it can not be regarded as a penetrating disinfectant. The layman believes that the more this disin-

fectant foams up when applied to a wound, the greater the disinfecting power. This visible action means that the hydrogen peroxide is being decomposed at the surface and that very little may reach the deeper recesses of a wound.

Boric Acid.—This is a very weak disinfectant if it possesses any activity at all. It enjoys a reputation as an eye disinfectant but has been shown to be practically valueless. Even in saturated solution it does not destroy common bacteria after a considerable length of time. The Bureau of Chemistry found it to be the main constituent of a canning powder which was advertised for use in home canned foods. This powder, containing 95 per cent of boric acid, was practically without effect on bacteria and its use might cause the slighting of other important steps in the canning technic. The fact that it was found to be inert on bacteria is the pertinent information here. Tanner and Funk (1919) made a short study on boric acid in disinfection. Their results indicated that boric acid is decidedly unreliable, as a disinfectant. Even in saturated solutions, it did not kill the common bacteria and many of the pathogenic types. Where it is absolutely essential to control the bacterial flora some other disinfectant should be used.

Ethyl Alcohol.—In comparison with other available compounds, ethyl alcohol is without much effect on bacteria. When it does act, its action may be explained as dehydration. The other homologues have also been studied and in the alcohol series one would have an excellent opportunity for studying the influence of molecular configuration on disinfecting power. The greatest disinfective power is secured in strengths of between 50 and 70 per cent. These solutions contain sufficient water for the coagulation of the protein. Beyer (1912) found that concentrations of alcohol under 60 per cent and over 80 per cent were practically worthless as disinfectants. Frey (1912) found that treatment with various concentrations of alcohol influenced the swelling and solubility of protein in water. Alcohol diluted to 10 per cent dissolved a little of the protein; above 20 per cent of alcohol solution of the protein stops and swelling decreases; above 90 per cent the protein dissolves. The greatest action of alcohol on protein is at 60 or 70 per cent while the greatest disinfecting power is 70 per cent. Frey explains the use of alcohol as a disinfectant in the irreversibility of the precipitation of protein after treatment in alcohol.

Glycerol.—Rosenau has made an interesting study of this compound in its relation to disinfection. He found that a 50 per cent solution of glycerol would restrain all bacterial growth and that lower percentages would allow better growth. Bacteria would not grow in media containing 32 per cent of glycerol but molds grew in stronger solutions of

40 and 49 per cent. Glycerol was found to have a distinct but slight germicidal action and probably acted by abstracting water from the bacterial cell. Spores were not killed and anthrax spores lived 200 days in strong glycerol solutions. The compound may be used as food by many bacteria. It is then probably oxidized to glyceric aldehyde and glyceric acid. Glycerol may be used, however, as a carrier for many other disinfectants such as phenol, cresol, etc.

Dyes as Disinfectants.—Recently it has been observed that dye-stuffs exert a distinct retarding action on bacteria. Churchman (1912) made an important contribution to this question. He reported that gentian violet in dilutions of 1 to 100,000 when added to media would inhibit the development of certain bacteria. The Gram-negative bacteria grew fairly well on the gentian violet media while generally the Gram-positive would not. The subject has been greatly enriched by much recent work especially that of Krumwiede and his co-workers. Some of these researches are mentioned in other places.

TABLE V
SHOWING ANTISEPTIC VALUES OF SOME COMMON SUBSTANCES

(After Park and Williams)

Alum.....	1 : 222	Mercuric chloride.....	1 : 14,300
Aluminum acetate.....	1 : 6,000	Mercuric iodide.....	1 : 40,000
Boric acid.....	1 : 9	Potassium bromide.....	1 : 10
Calcium chloride.....	1 : 143	Potassium iodide.....	1 : 10
Calcium hypochlorite.....	1 : 25	Potassium permanganate....	1 : 300
Carbolic acid.....	1 : 1,000	Pure formaldehyde.....	1 : 25,000
Chloral hydrate.....	1 : 333	Quinine sulfate.....	1 : 800
Cupric sulphate.....	1 : 107	Silver nitrate.....	1 : 12,500
Ferrous sulphate.....	1 : 2,000	Sodium borate.....	1 : 14
Formaldehyde.....	1 : 10,000	Sodium chloride.....	1 : 6
Hydrogen peroxide.....	1 : 20,000	Zinc sulfate.....	1 : 20

Soaps as Disinfectants.—Much misinformation on this question is held by the layman, who has been content with accepting the statements in advertisements. The assertion that soap solutions were germicidal was made by Koch, although his conclusions were not generally accepted. Soap may have value as an agent for removing bacteria mechanically but its bactericidal properties are probably very low. In order to improve their bactericidal properties various chemical compounds have been incorporated in many cases in the soap; this, in turn, has not given a product upon which much reliance as a disinfectant can be placed. Norton, of the University of Chicago, stated that the so-called germicidal soaps were no more useful than the ordinary soaps. It is better to purchase a good soap for cleansing and then follow it by

the disinfectant if a disinfectant is needed. Rosenau in his "Preventive Medicine and Hygiene" states, "Medicated soaps are for the most part a snare and delusion as far as any increased germicidal action is concerned." More recently, however, Walker has shown that soaps are not to be so easily relegated to a class of inactive substances in disinfection. He made soaps in the laboratory from chemically pure fatty acids and tested their germicidal properties against several microorganisms. The sodium and potassium soaps of the same fatty acids did not differ much in bactericidal properties. *Staphylococcus aureus* was extremely resistant to the soaps tested. *Pneumococcus*, however, was very sensitive to the action of the laurates, oleates, linoleates and linolenates, being killed in fifteen minutes by an approximately 1 : 50,000 solution of sodium laurate. The *Streptococcus* was also sensitive to the action of these soaps but *Eberthella typhi* (*Bacterium typhosum*) was resistant. Walker also studied the effect of four commercial soaps on the bacteria with which he worked. He stated that the susceptibility of *Meningococcus*, *Gonococcus*, *Streptococci*, *Pneumonococcus* and diphtheria bacilli was such that they were killed by any ordinary soap used with a reasonable degree of care. The dysentery bacilli, paratyphoid bacilli were killed with soaps prepared from the saturated fatty acids but were resistant to soaps made with the unsaturated fatty acids at ordinary temperatures. The best soap to use against these organisms was a so-called salt-water soap prepared with coconut oil. In interpreting Walker's data one must be certain to remember that his conclusions are based on laboratory experiments under conditions that would scarcely obtain in practical disinfection.

Much of the misinformation on this subject is due, perhaps, to malicious advertising. The booklet accompanying one of the most widely used soaps in the United States under a paragraph heading "Soap as an Antiseptic" contains this statement: "Physicians, trained nurses and surgeons depend on soap and water alone for protection against infection." The informed person who has given thought to the subject of disinfection will see the fallacious import of such a statement; the uninformed, however, will have no grounds for not believing it.

More recently Reasoner and Gill, United States Army Surgeons, have given soap another lift as a disinfectant. They stated that a good tooth soap would prevent trench mouth, or Vincent's angina. In this respect dentifrices which contain a good percentage of soap might be beneficial. We may leave the subject of soap as a disinfectant by saying that there are far better disinfectants.

Standardization of Disinfectants.—It is necessary to have some method of measuring the activity of a disinfectant. A chemical analysis,

even if it could be made, might not give much information about the disinfecting value of a compound. Consequently, it has been necessary to devise another scheme. This involves the determination of the *phenol coefficient* of an unknown compound. For this, some standard has to be taken as the **unit of disinfecting value**. This has been decided upon as the action of pure carbolic acid to phenol on a pure culture of *Eberthella typhi* (*Bacterium typhosum*). With this is compared the action of the unknown disinfectant on a pure culture of *Eberthella typhi*. Then the action of the known disinfectant, carbolic acid or phenol, on *Eberthella typhi* is compared with the action of the unknown disinfectant on *Eberthella typhi* and a coefficient is arrived at which numerically expresses the disinfecting value of the unknown disinfectant in terms of phenol. The phenol coefficient of phenol is 1.0, or lysol, 2.12, or tricresol, 2.62. The determination is usually made both in the presence and absence of organic matter.

Terminal Disinfection (Fumigation).—It has always been the custom to fumigate after a case of infectious disease. Different agents have been used, one or two of which will be discussed briefly.

Formaldehyde.—This is available on the market as formalin, a 40 per cent solution of formaldehyde in water. When this is diluted to make a 5 per cent solution of formalin in water it may be directly applied to materials. For fumigation, however, it is generally prepared in the gaseous state. We will not be concerned here with the methods. Dorset gave the advantages and disadvantages as follows:

- (1) It is one of the most powerful germicides known.
- (2) Its action is not interfered with by albuminous substances.
- (3) It is not poisonous and may therefore be used for disinfecting hay and grain without destroying these for food purposes.
- (4) It is not injurious to delicate fabrics, paint, or metals. (Formalin solutions will attack iron, but not other metals.)

The disadvantages are, briefly, as below:

- (1) The gas has a strong tendency to condense in cold weather and is not reliable as a disinfectant when the air temperature is below 50° F.
- (2) It is necessary to seal tightly all compartments which are to be disinfected with the gas in order that penetration may be secured and that the required concentration may be maintained for a sufficient length of time.

Formaldehyde changes to paraformaldehyde by polymerization when kept at room temperatures or lower. It unites easily with many substances, especially the organic compounds. For instance, when added to gelatin it forms a compound which is insoluble in many of the common

reagents. Fermi attempted to secure a gelatin medium in this way which would not melt at room temperature. It may act in the same way on bacterial protein. It is important to have plenty of moisture present when using formaldehyde as a disinfectant. Koch (1901) reported that a 0.05 per cent solution of this compound would kill growing yeast while a 0.005 per cent solution would not. Since carbon dioxide was formed after the cell had been killed, the zymase was not destroyed.

Sulfur Dioxide.—This gas, generated by the burning of sulfur, is a feeble disinfectant. It has been almost entirely replaced by formaldehyde.

Now we should analyze the real merits of fumigation as one of the objective methods for the spread of disease. Prominent sanitarians and students of the question are about agreed that terminal disinfection is without value. This statement may be verified by consulting such books as Park's "Public Health and Hygiene," and Rosenau's "Preventive Medicine and Hygiene." The reasons for not retaining terminal disinfection are:

1. It has very little effect on the control of communicable disease. In cities where terminal disinfection or fumigation has been abandoned, there has been no increase in communicable disease.
2. The methods and agents used have very little action, if any, on pathogenic microorganisms. These agents of disease might be imbedded in bits of organic matter and thus be protected from the action of the gaseous disinfectant. Pathogenic bacteria tend to die rapidly after discharge from the body.
3. It is much more important to destroy infective material throughout the course of the disease than to rely on one attempt at the end.
4. More active attempts to destroy infectious material should be made than are made in methods of terminal disinfection. A committee of the American Public Health Association which prepared a report on forty, or so, common diseases discussed terminal disinfection for each disease. In not one instance did this committee composed of eminent sanitarians advise terminal disinfection. After each case of disease, they recommended only *thorough cleaning*, defining this procedure as a generous use of hot water and soap suds.
5. The retention of terminal disinfection in public health work may tend to prevent the use of other procedures far more reliable. The layman may regard terminal disinfection as dependable and thus fail to apply other procedures.

The suggestion to abandon terminal disinfection caused considerable discussion. Those who have fought this proposal have argued that fumigation does no harm and might do some good. They have sug-

gested that the procedure be retained until a better one has been found with which to replace it. These arguments are of doubtful import since, if applied to other endeavors, would not be conducive to progress. Chapin, who has given the subject much discussion, made the following statement, "Which is the more likely to be the chief factor in the extension of contagious disease: inanimate objects hypothetically infected with dying bacteria or living or moving human beings who are continuously throwing off living bacteria?"

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CHAPTER XII

MUTUAL RELATIONSHIPS AMONG MICROORGANISMS

Having discussed the effects of physical and chemical agents on bacteria, attention may be given to the effect of microorganisms on one another. Cooperations and antagonisms are common among all forms of life. The terms which are discussed below as including most of the various degrees of relationship, may be illustrated by selecting some typical example from practical bacteriology. However, it is not easy to select an illustration which may be used exclusively for the purpose in hand. In some cases the distinction between terms is very close and may be even built up about a play of words. Pure cultures are necessary if the bacteriologist seeks fundamental information on the relation of one organism to another. Such cultures contain but one kind of organism or species. Since several methods have been developed for isolating single cells, pure cultures might be defined as cultures started from single cells. Mixed cultures, on the other hand, contain more than one kind of microorganism.

Symbiosis.—This is an harmonious and reciprocally beneficial relationship between two microorganisms or groups of microorganisms. Usually we like to believe that both organisms or groups are benefited, each contributing something to the other or helping to create and maintain conditions which are beneficial to the other. There are many kinds or gradations of symbiosis depending on the degrees of concord. Symbiosis may also exist between other forms of life. It is not a term restricted to bacteriology. Undoubtedly symbioses play an important rôle in nature's scheme.

Symbiosis between Bacteria.—Several illustrations may be offered. The growth of anaerobic bacteria together with aerobic bacteria is a typical example. The aerobic bacteria use up the oxygen and thus create favorable conditions for the development of the anaerobic bacteria which are unable to grow in the presence of oxygen. This association is, then, reciprocally beneficial to both groups.

Symbiosis between Bacteria and Plants.—Only the best-known illustration need be mentioned. It will be discussed more fully in a later chapter. It is the symbiotic fixation of atmospheric nitrogen. This

symbiosis is visually characterized by the formation of nodules or tubercles on the rootlets of certain plants. In these nodules develop certain bacteria which take nitrogen from the atmosphere and give it to the plant. In return for this nitrogen the plant gives the bacterium carbohydrates and probably other materials necessary in its metabolism. Another example might be the growing together of yeasts and acetic acid bacteria in the making of vinegar.

Symbiosis between Bacteria and Animals.—In the alimentary tract of animals are countless numbers of bacteria. While some may be there as transient inhabitants, others, such as *Escherichia coli* (*Bacterium coli*) take up their permanent abode there. They are spoken of as symbionts. They probably help to break down complex food materials and thus aid the digestive enzymes in the preparation of food for passage through the intestinal walls into the blood to be carried to the cells of the body. In the alimentary canals of animals, for instance, no cellulase is secreted and consequently the animals are unable to utilize the energy in cellulose. Cellulose is known to be fermented by bacteria in the alimentary tracts of animals. In this case the symbiosis is completed by the fact that the animal maintains a favorable place for the bacteria while the bacteria may make foods more readily available to the animal. Other beneficial effects have been suggested for explaining the presence of bacteria in the alimentary tract of animals but lack of space makes it impossible to discuss them. Since the vitamin hypothesis has become so firmly established, some have stated that the bacteria in the intestinal tract contribute vitamins to the animal. Are these bacteria strictly necessary for the normal well-being of the animal? This question would be a good one for the student to search out in the literature. *Commensalism* is another mutual relationship which should probably be mentioned. This relationship obtains when one organism lives on the waste products of another.

Antibiosis.—This relationship is just the reverse of symbiosis. In this case there is an antagonism between bacteria or groups of bacteria. It gives us an explanation in part, at least, why bacteria, multiplying as rapidly as they do, do not drive other forms of life from the earth. Among others, antagonism may be offered as an explanation for this. Bacteria cannot get along well together in some cases and the more hardy ones kill out the others. It is another illustration of the law of survival of the fittest. On what basis may antibiosis be explained? In some cases one group of bacteria may form large amounts of acid. Other bacteria may form products of metabolism which are objectionable to bacterial life.

Antibiosis between Bacteria.—The formation of acids, which has just

been mentioned, explains the antagonism between the putrefactive bacteria in the intestines and the organisms of the lactic acid group (*Lactobacillus acidophilus*) which are used to cure certain intestinal difficulties. The putrefactive bacteria cannot endure an acid environment. When lactic acid bacilli are established in the intestines by proper diet and feeding of the microorganisms, the putrefactive bacteria which cause illness are driven out. Acid-forming bacteria together with the lactic acid contained in their cultures have been advised for the clearing

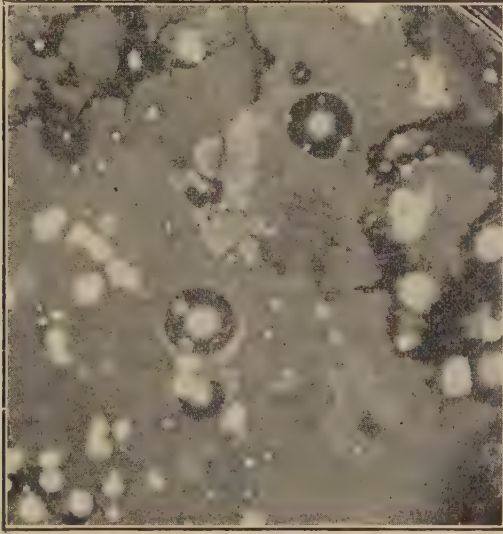


FIG. 98.—Showing Antagonism between Bacteria Growing on Plain Agar. Note the clear areas about the colonies where the growth of the spreading colony has been prevented.

up of diphtheritic throats. The lactic acid bacteria are believed to be antagonistic to the diphtheria bacilli.

Antibiosis between Protozoa and Bacteria.—Many protozoa feed upon bacteria; this is, indeed, the most extreme illustration that can be given—where one organism devours the other. This is made use of in the purification of water by filtration; the protozoa on the filters feed upon the bacteria which are caught on the filters. Protozoa are also responsible for the reduction in the bacterial population in natural bodies of water in the summer.

Parasitism.—This mutual relationship should also be discussed at this time. This term is of Greek origin and literally means a mess-mate and originally had no odium or contempt attached to it. The

meaning has gradually shifted until now it has assumed somewhat the significance of a "hanger-on," or one who gets his living from another. In this sense it has been taken over into biology and botany. Different degrees of parasitism are recognized. *Obligate parasites* are those which cannot grow away from living tissue or cannot be grown on laboratory media. *Facultative parasites* are those which may be grown on laboratory media. In contrast with the term *parasite* is the term *saprophyte*. The latter term is applied to an organism which can live on dead organic matter. The term *parasite* is a broad general term including both plant and animal life which dwell in or upon another organism called the host. The term, however, has been monopolized somewhat by the protozoologists who use it for parasitic animal forms. Morrey has contrasted these terms as follows. He stated that those bacteria whose food consisted of dead material, either organic or inorganic, are spoken of as saprophytes. On the other hand those bacteria whose natural habitat, irrespective of their food requirements, is in or on living organisms, are spoken of as parasites. He called attention to the fact that these terms are often used as if they were opposites, an erroneous position to assume. The term saprophyte has reference to food while the term parasite has reference to place of abode.

Pathogenesis.—This term comes from two Greek words meaning the origin of disease and is now used in the general sense to indicate development of morbid or disease conditions. It might be looked upon as parasitism where the parasite is more aggressive—does not simply secure its living from another living organism but, in addition, does harm to that organism. The subject is discussed more fully in the later chapters of this book.

Metabiosis.—This term is applied to a situation which is midway between a strict symbiosis and a strict antibiosis. It may be thought of as a condition where only one organism is actively benefited, the other receiving only passive benefit. It might be looked upon as a half-hearted symbiosis.

CHAPTER XIII

NUTRITION OF BACTERIA

The nutrition of single-celled microorganisms is a useful subject for study; the data arrived at help, in many cases, to elucidate the same problems for higher organisms with which it is often not easy to work. The higher forms of life are multicellular and consequently their metabolism rests upon more intricate factors. In this chapter a broad view of metabolism will be assumed in order to introduce the metabolic characteristics of both plants and animals. This will give a better foundation for understanding the metabolism of the unicellular microorganisms.

Amount of Food Required by Bacteria.—This factor is quite variable. It depends on many different conditions just as it does with the higher organisms. We need not be concerned with the detailed discussion of this question but some profit will result from a consideration of the small amounts of food required by microorganisms. This is best appreciated by considering the size or weight of an ordinary bacterial cell and the amount of solid matter contained in it. This will strikingly illustrate the food and energy requirements of a cell. In a former paragraph we used Kendall's data and statements about *Vibrio comma* (*Microspira cholerae*) to indicate the possible number of progeny from a cell. We will also take his data and statements to determine how much food a cell of this organism would require. An average cell of this organism is about 1 micron (0.001 mm.) in diameter and 2 microns (0.002 mm.) in length. Such a cell would weigh about 0.000,000,002 mg. assuming that the specific gravity is 1. This means that 1,000,000 of the cells would weigh only 0.2 mg. Then, assuming that 80 per cent of the bacterial cell is water, it is evident that in 1,000,000 cells there would be only 0.04 mg., an extremely small amount of solid matter in 1,000,000 cells. This amount is hardly measurable and when one tries to comprehend the amount of solid matter in one cell, it is difficult.

What Is Food?—For the higher organisms food may be defined as material which, when taken into the body and properly prepared by the body fluids, may be utilized for the repair of old tissue, the building of new tissue and the generation of energy. This definition suffices, also, for the bacteria, although we lack detailed information of the

mechanisms by which the food is made available to the single-celled bacterium. Their small size makes it difficult to determine some of these facts.

Foods for Growth and Building Purposes.—Foods are classified in many different ways depending on the purpose of the classification. Ordinarily they are classified as proteins, fats and carbohydrates. They may also be classified on the basis dialyzability. For our discussion, they will be separated into two groups, *food for energy* and *food for growth*. Such a separation is of course an arbitrary one. The proteins or nitrogenous compounds are generally offered as good examples of foods for growth and the carbohydrates as foods for energy purposes. It would be wrong, of course, to assume that the fragments of a carbohydrate molecule, broken up by fermentation, could not be used for building and repair or just as wrong to deny that the decomposition of proteins yields energy.

Metabolism, Katabolism and Anabolism.—Metabolism is the broad term including katabolism and anabolism. The processes of metabolism and nutrition are said to consist of the conversion of food into body tissue and both food and body material into energy. It is then a change of potential energy into kinetic energy.

Anabolism (Assimilation).—This term covers the synthetic or building processes in the cell. The reactions involved are endothermic reactions requiring energy. The energy which is secured by fermentation is utilized for building purposes. Anabolism, of course, is the foundation of growth.

Katabolism (Dissimilation).—The processes involved in and included under the term katabolism are decompositions. They are sometimes spoken of as analytic reactions. As with other cell functions katabolism is best explained as an enzyme phenomenon. Katabolism must not exceed anabolism else the cell will be killed. There may be times, however, when anabolism is reduced to a minimum and katabolism progresses more rapidly. This results in a wasting away of the cell.

Digestion.—This term may be discussed here mainly for the sake of completeness. It includes those processes by which the foods (in the alimentary tract of animals) are reduced in complexity and made able to pass through the walls into the cell. In the strict sense, then, digestion goes on outside of the cell. Under our discussion of enzymes we will discuss this term more fully. The action of extra-cellular enzymes secreted by microorganisms to make foods permeable might be looked upon as digestion even though the preparatory changes do not take place in a digestive tube as they do in higher animals. The food in the digestive tubes of higher animals is not inside of the body since the ali-

mentary canal is just a tube extending through the body. In the alimentary tracts of animals, the non-permeable foods are made permeable by means of hydrolyzing enzymes. This change is quite analogous to the action of extra-cellular enzymes in bacterial metabolism.

Following the hydrolysis in the animal alimentary tract, absorption must take place. The products of hydrolysis must pass through the intestinal wall into the blood which carries them to the cells.

Plant Metabolism.—The metabolism of the green plants is sharply set apart from that of the animals and fungi. The green plants possess chlorophyll by means of which they are able to utilize the energy in sunlight and with it to build up their complex body constituents by means of a process called *photosynthesis*. This process is endothermic, meaning that energy is used up in putting together CO_2 and H_2O to make proteins and carbohydrates. This energy comes from the sunlight. We might say that it is changed from kinetic to potential energy. Each molecule of carbohydrate, protein, or fat is then virtually a storehouse of energy for organisms which cannot utilize sunlight but which must resort to the chemical energy latent in large molecules. The subject is fully treated in books on Plant Physiology.

Animal Metabolism.—Animals are devoid of chlorophyll and therefore cannot resort to sunlight for energy. They have to go to the storehouses of energy constructed by plants and secure it by decomposition of proteins, fats and carbohydrates. They resort to chemical decompositions and consequently we may speak of this energy as *chemical energy* in contrast with *solar energy* used by plants. In the strict sense, then, at least for the present discussion, the ultimate source of energy is the sun.

Bacterial Metabolism.—Bacteria also are unable to use solar energy and therefore have to use chemical energy. In the next paragraph the relation of surface area to body weight will be discussed. On account of the ratio which exists in an average bacterium, much more energy has to be provided by bacterial metabolism. On account of the absence of chlorophyll in bacterial cells, their metabolism is more like that of some of the lower animal forms.

Foods for Energy.—The energy requirements of bacteria are interesting. The bacterial cell, taking *Vibrio comma* (*Microspira cholerae*) ($1\mu \times 2\mu$) as an example, would have a surface area of 0.00001 square millimeter. Since the specific gravity is about 1, the ratio of surface area to weight of bacteria is 1 to 2000. Kendall¹ stated that for comparison a man 200 cm. tall, weighing 75 kg., had a surface area of about 2 square meters. Then, the ratio of surface area to weight is much

¹ Kendall. Colloidal Symposium Monograph, p. 195.

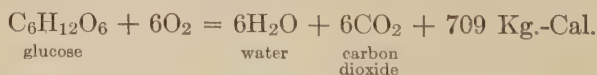
nearer unity in man than with bacteria. Since the energy requirements of living organisms varies with the surface area, bacteria will require more energy than man.

Erwin F. Smith discussed this question as follows:

The reason the bacterial cell accomplishes work out of proportion to its size is just this, that its oxygen-absorbing surface is enormously greater in proportion to volume of protoplasm than that of any other known organism. The surface of the rods in a cubic centimeter of bacterial slime, such as we frequently obtain in a test-tube on our solid media and observe in the plant, represents an oxygen-absorbing area equal to the surface of an ox. Indeed, we might say that the smallest bacteria are almost all surface.

This helps to explain why the bacteria are so active in food decompositions and certain industrial fermentations such as the acetic-acid fermentation, the acetone-butanol fermentation, etc.

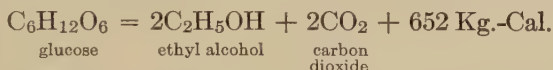
Respiration.—In the early days of the science of bacteriology it was believed that free oxygen (atmospheric oxygen) was necessary for all forms of life. This was later shown to be too general a statement and several terms were introduced into discussions of this subject to cover the different gradations and types of oxygen utilization. The term respiration is used by plant physiologists to indicate the oxidation of reserve organic substances. It is, then, quite similar to combustion. The oxidation of these materials in the plant yields energy just as its combustion outside of the body would liberate heat (energy). Normal respiration, then, may be regarded as a process dependent on and involving the use of free oxygen. It may be replaced by fermentation which also liberates energy. Respiration tends to result in completely oxidized products and therefore allows the formation of more energy since less is left in the end products. The following equation might be taken to illustrate respiration.



Aerobic Respiration.—The term *aerobic* was introduced in 1861 by Pasteur for describing those bacteria which could use free atmospheric oxygen or required it in their metabolism and multiplication. On account of having plenty of available oxygen, aerobic respiration often results in complete combustion of the food material. The equation given in the above paragraph is a good example. The products of aerobic respiration are usually completely oxidized products and the microorganism has been able to drain the large molecule, such as glucose, of all of its energy, leaving fragments which are inactive.

Anaerobic Respiration.—This term was introduced by Pasteur to describe bacteria which did not require atmospheric oxygen in their life processes. Indeed the organism (*Vibrio butyrique*) not only could get along without free oxygen but was poisoned by it. Such a statement coming at a time when every one believed that atmospheric oxygen was necessary caused much discussion and argument. Pasteur found that free oxygen poisoned these cells and caused them to lose their motility. Consequently the evil effects of free oxygen were demonstrable by examining a drop of a culture of this organism under the microscope. At the edge of the drop where oxygen diffused into it, the organisms were non-motile, but at the center they maintained active motility. The organisms which could not use free oxygen and were harmed by it were spoken of as *anaerobic* and the process as *anaerobiosis*.

From the standpoint of energy liberation anaerobic respirations are less efficient. Since they are carried out in the absence of free oxygen complete oxidation does not occur. We might use an anaerobic decomposition of glucose to illustrate this.



The fact that along with the CO_2 are formed two molecules of ethyl alcohol indicates that some of the potential energy in the glucose molecule will remain in the ethyl alcohol. By subtracting the number of calories formed in this equation from the number formed by complete combustion, we find that only 57 Kg.Cal. are available to the bacterial cell that resorts to anaerobic respiration.

Aerobic, Anaerobic and Facultative Anaerobic Bacteria.—Before leaving this subject, since several new terms have been introduced let us make certain that they are clearly defined. *Aerobic bacteria* are bacteria which can use only free atmospheric oxygen. *Anaerobic bacteria* are inhibited, if not killed by free atmospheric oxygen. They are able to get the oxygen which they need from more or less completely oxidized compounds. Between the strict aerobes and anaerobes is a group of organisms which are more or less indifferent to oxygen. Those anaerobes which, while preferring strictly anaerobic conditions, are able to live in the presence of free oxygen, are called *facultative anaerobes*.

Intramolecular Respiration.—This is another view that may be taken of respiration. It really signifies those rearrangements, involving a shifting of oxygen within large molecules, by which energy is liberated for the use of the cell. These large molecules may be fragmented

and, while considerable energy is retained in the incompletely oxidized products, enough is liberated to make the decomposition of profit to the cell.

Intermolecular Respiration.—This term is applied to respiratory processes which involve two molecules. The oxygen is taken from one molecule and carried over to another. The process of removing oxygen from one molecule may cost the organism some energy, since it may be an endothermic reaction, but the cell capitalizes on this investment when it uses this oxygen for breaking down another compound. There must be a net return to the cell, for no cell could go on indefinitely with decompositions which did not yield anything to the cell.

Fermentations.—These are often defined as decompositions, usually of carbohydrates, which result in the liberation of energy. Fermentations may take the place of respiration. They are usually cleavages of large molecules into simpler ones. Pasteur defined fermentation as respiration without oxygen—apparently meaning free oxygen. We now know that bacteria ferment in absence of free atmospheric oxygen and take oxygen from combined sources. Fermentations may then be looked upon as anaerobic respirations. Fermentation may also be looked upon as an intramolecular respiration.

Fermentations are generally known by the predominant product such as alcohol, acetic acid, lactic acid, etc. One can scarcely think of a process which has more significance and interest than fermentation.

Autotrophic, Prototrophic and Heterotrophic Bacteria.—Microorganisms may be divided into three groups depending on the kind of compounds used. *Autotrophic* organisms use the element in the inorganic conditions. A *prototrophic* organism uses the uncombined element itself and a *heterotrophic* organism uses the element in any form. In looking over the known species, we readily see that most of them are heterotrophic with regard to each of the more common elements. Under the discussion of the fixation of atmospheric nitrogen we will discuss *prototrophic* bacteria. Autotrophic bacteria are those which live in inorganic media.

Mineral Foods.—In a former paragraph, the weight of a single cell of a typical bacterium was computed. It was found to be very small. When it was pointed out that only 15 or 20 per cent of this was solid matter the figure became still smaller.

There is no reason for not believing that the same elements which are necessary for higher forms of life are not necessary for the bacteria and closely related organisms. These are calcium, potassium, phosphorus, sulfur, magnesium, iron, etc. In carrying out experiments to determine whether certain elements are necessary or not, it is difficult to exclude

traces which adhere to apparatus or are in the air. Statements, then, to the effect that these compounds are not needed should be accepted with reservation. Mineral foods are often supplied to bacteria in what are called synthetic media. A better name might be had in mineral salt-sugar solution, in which sugar is offered as the source of carbon.

Organic Foods.—Most of the decompositions that have been discussed in this chapter are those of organic compounds. On this account we need not give much more attention to them. An interesting question in this connection is whether bacteria can live indefinitely away from organic matter and carry out their complete life cycles for indefinite generations in inorganic media. Answers to the question are influenced by a number of factors. We can enumerate several illustrations. The nitrate bacteria, those which oxidize nitrites to nitrates, are believed to live without organic carbon; they are cultivated in a medium in which the only carbon is in the inorganic state as sodium carbonate. It is possible, however, that they secure organic carbon from the atmosphere. The same is probably true for the nitrite bacteria, those oxidizing ammonia or its compounds to nitrites. The vitamins are also in the class of organic foods. The question as to whether they are necessary or not is another interesting one.

Water Requirements.—Water is necessary in the metabolism of living organisms for a number of reasons. It cannot be regarded as a food but does act as a conveyor of foods. It is necessary for the conveyance of foods through the cell wall into the cell and for the removal of the waste products from katabolism from the cell. The necessity for water has been considered in several other places also.

CYCLES OF THE ELEMENTS

It has become customary for bacteriologists and agriculturists to discuss the nitrogenous metabolism, the sulfur metabolism, the phosphorous metabolism, etc., of microorganisms, with the help of an orderly system called a *cycle*. Thus we may have a *nitrogen cycle*, a *carbon cycle*, a *phosphorus cycle*, etc. We will retain this useful method for presenting the facts of nitrogen, sulfur, and phosphorus metabolism of the bacteria. It gives us a fine method for following the various stages through which compounds of elements pass. It will also help us to correlate our information.

The soundness of this practice rests upon the fact that matter is indestructible as is expressed by the Law of Conservation of Matter. Russell and Hastings in their "Agricultural Bacteriology" have stated it in this manner:

An atom of carbon may be in the atmosphere to-day in the form of CO_2 ; to-morrow it may be in a sugar molecule of a plant; the next day in the tissues of an animal; and the succeeding day it may be again present in the air in a molecule of carbon dioxide; ready for another of its ceaseless passages, carrying with it a supply of energy for the animal and fungus plant.

Cycles then depict the various states of oxidation of elements in an orderly manner. In one or two of the following chapters the nitrogen and sulfur cycles will be taken up.

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CHAPTER XIV

GROWTH OF BACTERIA

What is Growth?—With the higher animals and plants it is not difficult to determine whether an organism has grown or not. This may be determined by weighing to secure the increase in body weight in pounds or ounces, or it may be done by measuring the increase in size. Microbiologists cannot resort conveniently to such methods although there are special methods by which this is done. The microbiologist has had to adopt a different conception of growth not always appreciated by those who are not trained in microscopic technic. The conception of the term growth given above—that it signifies increase in weight or cell mass—may be retained by microbiologists. However, *multiplication*, an increase in numbers of individuals, should be distinguished from growth. Thus, cells may grow after multiplication as well as just before. Work with yeasts has shown that they may grow to a considerable extent after multiplication. The term reproduction is also used to express increase in mass or numbers. Strictly speaking, it refers to the generation of new organisms from similar ones which have gone before. It depends on the division of the cells. It is apparent that several terms, used to express increase in mass, numbers or volume, overlap considerably and may be defined in different ways.

Methods Used for Measuring Growth, Multiplication, etc.—Having attempted to define these terms, some attention may be given to methods which are used for measuring the phenomena which they represent. Confusion has resulted in microbiology from misinterpretation of data.

Counting of Plate Colonies.—The uninformed layman wonders how bacteriologists are able to report such great numbers of bacteria in foods as milk, ice cream, etc. Buttermilk, for instance, may contain as many as 100,000,000 bacteria per cubic centimeter. To secure this content of bacteria, the bacteriologist does not count each cell. The original material (milk, for instance) is diluted with sterile water to such an extent that the number of bacteria in 1 cc. may be counted. This number or count is then multiplied by the dilution factor to secure the total number. Since by this method, we count single cells or clumps which have developed into colonies, it is really a measure of multiplication.

Counting of Individual Cells.—Another method of counting involves the use of a chamber called a hemacytometer originally devised for counting the red and white corpuscles in the blood. A small portion of the material, the bacterial content of which is to be ascertained, is placed on a very finely ruled surface. This is then placed under the microscope and, knowing the area of the squares, the number of bacteria in the milk may be determined. This method also is really a measure of multiplication.

Measuring the Volume Consumed by the Cells.—By this method the bacterial cells in a material are thrown to the bottom of a special tube on a centrifuge. The volume of the precipitate gives information with regard to the content of bacteria. It is obvious that data secured by this method are different from data secured by the methods just described.

Measuring Bacterial Content by Estimations of Turbidity.—This method is, of course, applicable to clear media only or to solutions the turbidity of which is due only to the bacterial cells. This method requires an instrument known as a nephelometer. Data collected by this method, of course, depend on both multiplication and growth. The more cells and the larger they are, the greater the turbidity. This method is used for estimating the number of cells in a saline suspension for the preparation of bacterins (bacterial vaccines).

Growth Histories of Cultures.—The growth history of a culture centers about the numbers of cells at different ages. We need mention but three stages or phases, the lag phase, the phase¹ of rapid growth and the last phase spoken of by some as the phase of decreasing growth or that in which the cells are dying.

The Lag Phase.—This is the phase immediately following the inoculation of the fresh sterile medium when the culture is started. During this phase the bacteria do not multiply but remain constant in numbers or even decrease in numbers. During this phase the cells are probably becoming adjusted to conditions in the new medium.

Phase of Rapid Growth.—This phase follows the lag phase. During this phase the cells are dividing with the shortest generation time when the best conditions of food concentration, temperature, etc., are obtained.

Phase of Decreasing Numbers.—A culture eventually reaches a point where the number remains constant for a time to later decrease. What is responsible for this? Why do not the cells in a culture continue to grow indefinitely? Two reasons are generally given. These, discussed

¹ The several phases mentioned here are a few taken from a greater number which are recognized in the growth of bacteria. The others are discussed by Buchanan, Jour. Inf. Dis., 23 (1918), 109-125.

below, will be referred to again in connection with two early explanations of immunity.

Lack of Food.—Multiplication in a culture may stop on account of lack of food. This has been studied by several investigators and found to explain the situation. Graham-Smith found that the addition of fresh food to cultures which had already stopped multiplying would cause multiplication to begin again.

Accumulation of Products of Metabolism.—All living animals require food and give off waste matter spoken of as “products of metabolism.” While there are some data to support this explanation for the cessation of growth or multiplication in a culture, the one offered above has more to support it.

Growth Histories of Single Cells.—The separate cells in a culture also pass through a series of stages which may be distinguished from one another. These stages have not received much consideration until more recently. Recent investigational work indicates that the members of a culture go through three stages in their development—a period of adolescence, a period of maturity and finally a period of senescence. During each of these periods the shape of the cell may be quite different from that in the others. These stages have been found to follow one another in regular order. Lack of appreciation of this caused earlier workers to introduce the term “involution form” for the shapes which were different from what were believed to be normal forms.

Rate of Growth.—The rate of growth, as would be expected, is dependent on a number of different factors. Some of these are discussed in the next paragraph and here we will consider some of the available data. In gathering these data the following have to be determined: the number of bacteria at the beginning, the number at the end and the time. If we allow the number at the beginning to be represented by a , the number at the end by b and the number of generations by n , we would have the following equation,

$$2n = \frac{b}{a}.$$

Then if G is the time required to complete a full generation,

$$G = \frac{t}{n}.$$

The validity of data collected with this equation depends upon the assumption that each cell divides into two parts and that all the cells in a culture act just alike. Barber studied this question with *Escherichia coli* (*Bacterium coli*) and found a generation time of seventeen minutes

at 38° C., under probably the best conditions. On both sides of this temperature, the formation of new generations required more time. Other workers with other organisms secured data not very different from these. Perhaps, the general conclusion might be reached that, under optimum conditions that exist in experimental work, the generation time is between thirty and forty minutes for ordinary bacteria. The ideal conditions probably do not exist in nature and the generation time would be much longer. The rate of multiplication would, of course, vary for each species. Kendall stated that *Vibrio comma* (*Microspira cholerae*) could form a new generation every fifteen minutes. Since there are 96 periods of fifteen minutes in each day of twenty-four hours, the theoretical descendants of a single bacterial cell would be 2^{96} or to express this another way, the descendants of one cell at the end of one hour would be 16 and at the end of two hours, 256; at the end of twenty-four hours the theoretical number would be 8×10^{28} . Such a number of descendants, however, is scarcely ever attained by a single organism in nature, for the factors influencing growth probably retard multiplication and cause the death of many cells.

Factors Influencing Growth and Multiplication.—Growth and multiplication are complicated processes. They represent the algebraic sum of the processes which go on in a cell.

1. *Concentration of Food.*—Under ordinary conditions this is an unimportant factor. It has been stated elsewhere that bacteria need only a very small amount of food. They will grow quite well in distilled water, which contains such a small amount of matter that it cannot be detected by ordinary chemical methods. However, under such conditions growth stops more quickly than when greater amounts of food are available.

2. *Hydrogen-Ion Concentration.*—Bacteriologists have always known that the reaction of media was one of its most important characteristics. Until the present methods of determining hydrogen-ion concentration were provided by the physical chemist, the bacteriologist had to resort to titration methods with N/20 hydrochloric acid or N/20 sodium hydroxide. As information accumulated, it was apparent that these procedures did not give an accurate picture and that such a factor as reaction (acidity or alkalinity) could be better measured by means of hydrogen-ion concentration determinations. For many of the common organisms the maximum, minimum and optimum hydrogen-ion concentration has been determined by various investigators.¹

¹ No explanation of the meaning of the term "hydrogen-ion concentration" will be attempted here. It is given in the author's "Practical Bacteriology," John Wiley & Sons, Inc., New York, 1928.

TABLE VI

HYDROGEN-ION CONCENTRATION RELATIONS OF SOME OF THE COMMON BACTERIA

Name of Organism	Hydrogen-Ion Concentration Relations	
	Limits	Optimum
<i>Corynebacterium diphtheriae</i>	6.8-8.3	7.3-7.6
<i>Mycobacterium tuberculosis</i>	6.0-7.6	6.8-7.2
<i>Escherichia coli</i>	4.6-9.5	5.2-8.4
<i>Serratia marcescens</i>	5.8-8.0	6.0-7.0
<i>Bacillus anthracis</i>	6.8-8.5	7.0-7.4
<i>Clostridium tetani</i>	5.5-8.3	7.0-7.6
<i>Clostridium botulinum</i>	6.0-8.2	7.0

3. *Temperature*.—This is another important influencing factor on growth. Since growth is composed of numerous chemical reactions or, at least, depends on them, it is not surprising that temperature has an important effect. The temperature relations are important characteristics of all bacteria. They constitute one of the important differential characteristics by which microbiologists are able to differentiate pure cultures. The driving effect of temperature on growth and multiplication was recognized by the early students. We have given considerable space to this factor on former pages. As we have already learned, the bacteria may be divided into three groups on the basis of their temperature relations; attention was called to the maximum, optimum and minimum temperatures. It will be unnecessary therefore to recapitulate this information.

It is not easy to discuss this important factor in a few paragraphs. Some pioneer work on the influence of temperature was carried out by Ward, the great English botanist. He found that at the optimum temperature, the growth was very rapid and lasted for a long time; at this temperature the organism was able to use its food to best advantage and give the greatest growth or "crop." However, above the optimum, while at first the growth was very rapid, it lasted for a shorter time. This decreased growth continued in effect as the temperature was raised until finally a temperature was reached where no growth occurred at all.

4. *Available Moisture*.—Water is necessary for all forms of life; it is just as important for the bacterial cell as for higher organisms. However, it seems that the bacterial cell is able to endure the absence of water more easily than higher animals or plants.

5. *Accumulation of Waste Products.*—Why do bacteria stop growing in a culture? Why do the cells not continue to grow until they have filled the medium and caused it to lose its properties of a liquid and become a gel? Search for information with which to answer this question has resulted in several answers. Some of the earlier investigators from experiments, the details of which need not be reviewed here, suggested that toxic substances were formed. The name *autotoxins* were given to these substances. Another suggestion was that peroxides were formed which, as they increased in concentration, retarded growth or multiplication. Another theory has it that the retardation is due to the formation of acids and a lethal hydrogen-ion concentration. Perhaps none of these explanations is true; perhaps all of them play a rôle. Investigational work is being continued and it is possible to anticipate more information.

6. *Vitamins.*—Interest in these accessory substances to the diet of higher animals has stimulated investigators in the field of bacteriology to find out whether their microorganisms needed such substances. The information secured with the microorganisms has resulted in a situation just as confused as that on the relation of vitamins to the development of animals. Much of the confusion in our own field has resulted from the inability or unwillingness to make any distinction between accessory substances and stimulating substances. An accessory substance might be regarded as one which is absolutely necessary for normal multiplication or growth. A stimulating substance might be considered as one which is not strictly necessary in the diet but which, when present, stimulates the growth or multiplication. For completeness we may mention three groups of accessory substances which have been, at various times, proposed in the literature for various forms of life.

Vitamins: Materials of unknown constitution necessary in the diet, other than fats, proteins or carbohydrates, or mineral salts, for animals.

“Bios” Substances of unknown composition supposed to be necessary for the best growth of yeasts.

Auximones: Substances of unknown composition supposed to be necessary for the growth of plants.

7. *Available Amino Acids.*—In studies on the growth and normals development of higher animals, biochemists and biologists have found that certain foods are deficient because they lack certain amino acids. For instance, gelatin containing no cystine or tryptophane is inadequate and must be supplemented by these amino acids or material containing them. The real purpose for the use of peptones for the preparation of

bacteriological media is to supply the various amino acids. The peptones and proteoses, chemically speaking, play only a secondary rôle for most of the common bacteria.

8. *Surface Tension*.—This is one of the more recent factors which has been found to influence growth. Ordinary culture media such as plain broth, according to Larson, have a surface tension of 59 dynes. When this was reduced to 32 dynes by the addition of certain surface tension depressants, some interesting alterations in growth were noticed. *Bacillus subtilis*, which ordinarily grows with a pellicle, was found to grow down in the medium when the surface tension was reduced to 45 dynes or below. The details of the effects of surface tension may be left for future study.

CHAPTER XV

BACTERIAL ENZYMES

Much of the present-day information on metabolism is based on the action of enzymes. These agents are very convenient explanations for many changes brought about by living cells. While there are many established data, it is probably true that the enzyme theory of cell activities is a very convenient refuge for uncertainty. It is probably top-heavy, for any new cell activity is often conveniently, if not correctly, explained by hypothecating the existence of a special enzyme. However, certain enzymes are sufficiently well known by their actions to justify consideration. Some of them have assumed important rôles in industrial processes.

Proof of Existence of Enzymes.—A number of observations caused early microbiologists to seek information which resulted in the discovery of enzymes. One of these prepared a sterile extract of malt which converted starch into sugar in the same manner as did strong acids. This extract contained no living cells so far as could be determined and the change which it brought about had to be explained on the basis of some other agent than living cells.

Extracellular Enzymes.—The existence of extracellular enzymes may be shown by growing bacteria in media and filtering them after good growth has occurred, to remove bacterial cells. The enzymes which have been secreted by the cell are present in the medium about the cell. They pass through the sterile filter and appear in the filtrate.

Intracellular Enzymes.—These were definitely demonstrated by Buchner in 1897. He worked with fresh brewers' yeast; it was carefully washed, mixed with sterile quartz sand, and ground in a mortar. This ground mass was then transferred to a press cloth and subjected, in a filter press, to a pressure of 90 kilos per sq. cm. The juice which contained the enzyme was carefully collected and filtered through an earthenware filter to sterilize it. The action of this sterile filtrate was then tested on the sugar. If zymase was present, the sugar was fermented. To prove definitely the existence of intracellular enzymes, the following procedures must be carried out.

1. Crushing of the yeast cells or grinding with some materials such as sand.
2. Filtration of resulting yeast juice through sterile filters which will remove the living yeast cells.
3. Proving the absence of cells in the sterile filtrate to which fermentation might be attributed.
4. Testing the action of the zymase on sugar to determine whether it would incite fermentation.

Nomenclature of Enzymes.—These agents were called ferments in the early days of bacteriology; two types were mentioned, *organized ferments* and *unorganized ferments*.

Organized ferments were the living cells of the fermentation micro-organisms. Consequently they could be seen under the microscope and demonstrated by any of the culture methods.

Unorganized ferments were not living cells but agents contained in living cells which were responsible for the changes brought about by living cells. They are known now as *enzymes*.

The distinction was of course arbitrary; as knowledge increased it became less and less tenable. The term ferment was introduced to indicate the agent which could be prepared by extracting certain substances and which would bring about the same reactions as the living cells. When Pasteur proved that fermentation was due to living organisms, the above distinction was made. Kühne, in order to avoid the use of these confusing terms, proposed the term *enzyme* (meaning in yeast) for these bodies.

The system by which enzymes are named was proposed by Duclaux and involves the use of the name of the substance decomposed, which is spoken of as the *substrate*. The name of the enzyme is made from the name of the substrate by substituting the ending *ase* for the last part of the name. In order to illustrate this method, a few examples may be given.

<i>Substrate</i>	<i>Enzyme</i>
Lactose	Lactase
Maltose	Maltase
Proteins	Protease

While this is the generally accepted method of naming enzymes, the student will often encounter the name of an enzyme which was not named in this manner. Some of our oldest and best-known enzymes were not named in accordance with Duclaux's suggestion, since these names were given before any system was regarded as necessary. Such are rennin, pepsin, trypsin, erepsin, etc. The last-mentioned names may, however, be changed to comply with the system of nomenclature suggested by Duclaux.

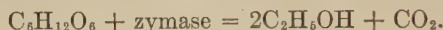
In order to distinguish enzymes which are mainly analytic for which the ending *ase* is used, from those which are mainly synthetic, it has been suggested that the ending *ese* be used for the latter.

Chemical Changes Brought about by Enzymes.—The types of chemical changes accomplished by enzymes are varied because an enzyme may be suggested for every change brought about by bacteria. Some of the more important changes may be tabulated as follows:

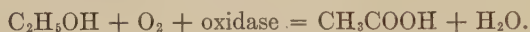
I. Hydrolysis = addition of water followed by splitting.



II. Splitting = division of the molecule without the addition of water.



III. Oxidation = addition of oxygen.



IV. Condensation = synthetic = union of carbon atoms.

V. Reduction = removal of oxygen.



VI. Coagulation (probably in most cases an hydrolysis).

Intracellular and Extracellular Enzymes.—From the standpoint of physiology of the single-celled organisms, the division of enzymes into extra- (outside) and intra-cellular (inside) enzymes is an important one. If the student understands the functions of both groups, much has been learned about the metabolism of bacteria and also the higher organisms.

It is well known that certain food cannot pass through a membrane such as the bacterial cell wall but yet these foods are utilizable by the bacterium. To allow them to be used they must be broken down to compounds which dialyze and pass into the cell. A food to be of any use to a cell must get inside of it. So that is the purpose of the extra-cellular enzymes—to break down (by hydrolysis) large molecules which will not dialyze to smaller ones which will. The energy liberated by these agents of the cell is of only indirect value to the cell. It is not formed in the cell and is therefore not available. It may go to warm up the medium slightly. The actions brought about by the extracellular enzymes are analogous in some respects to those of digestion in animals. Animals possess an alimentary tract, into which is placed a great amount of food. Such food is not yet in the body, for the alimentary tract may be considered as a tube running through the body. Food in this state cannot pass through the walls of the intestines; it must be hydrolyzed to simpler chemical compounds sometimes spoken of as soluble com-

pounds, although the term soluble has a different meaning here than it usually has. It refers to ability to pass through the walls of the intestines. In order to bring about this hydrolysis, digestive juices, which contain extra-cellular enzymes, are poured into the alimentary canal. These enzymes are peptase, tryptase, and ereptase. They break down the proteins to simpler compounds, amino acids; the latter dialyze through the intestinal wall into the blood which carries them to the cells. After they are taken into the cell another set of enzymes acts.

The intracellular enzymes, on the other hand, as the name indicates, do not pass out of the cell but remain inside and bring about those vital changes which are so necessary in the cell's life. The energy needed by the cell is gained from these internal reactions as are also the foods for construction of new tissue in growth and multiplication. Thus it is evident that the intracellular enzymes bring about necessary decompositions.

The relationship of these two groups is illustrated by the following steps in the decomposition of starch by enzymes.

1. Amylase changes starch to maltose.

2. Maltase changes maltose to dextrose.

3. Dextro-zymase changes dextrose to alcohol.

4. Alcoholase changes alcohol to CO_2 and H_2O .

The first two changes are brought about outside of the cell by means of enzymes. Starch must be broken down to soluble compounds because it cannot enter the cell in its original state as starch. The last two changes occur inside of the cell and are, therefore, the useful and important reactions.

How Do Enzymes Act?—This question may not be accurately answered although several theories have, at various times, been proposed. One of the most tenable explanations states that the enzyme unites in some manner with the substrate. An intermediate compound is formed which breaks down later liberating the enzyme again. During this contact with the enzyme the substrate is changed or broken down. There is no agreement with regard to how this union takes place, whether it is a chemical union or a physical one. According to this explanation, then, the enzyme is a sort of carrier with which succeeding loads of substrate are broken down.

Characteristics of Enzymes and Enzyme Reactions.—It is difficult to formulate a good definition of any enzyme. We know them by what they do rather than by what they are. Enzymes and enzyme reactions have the following characteristics.

1. Enzymes are organic catalysts.—The term organic in this sense sig-

nifies that they are connected in some way with living matter. They are not alive themselves but are formed by living cells. No one has been able to synthesize an enzyme nor has any one been able to analyze one to determine its chemical structure. Enzymes are also like catalysts in that their speed of reaction is increased as the temperature is raised. Some agents greatly stimulate the activity of an enzyme. They are called activators. Those which inhibit enzymes are called inhibitors.

A *catalyst*, according to Ostwald, is any substance which, without itself appearing in the final product of reaction, alters the velocity with which that reaction takes place. It should be emphasized that a catalyst does not start reactions, it merely speeds up those which are going on so slowly that the rate cannot be measured.

2. *Enzymes are Specific in their actions.*—This is one of the most interesting things about an enzyme. It helps to differentiate an enzyme from an inorganic catalyst such as palladium black, etc. Fischer used the lock and key analogy for illustrating enzyme specificity. The substrate was considered as the lock and the enzyme as the key. It takes a certain key to turn each lock; similarly it takes a certain enzyme to decompose each substrate. Each enzyme seems to have only one task to do and it will not assume others.

3. *They produce a great amount of reaction.*—This is another characteristic which puts enzymes in a class by themselves as active agents. They produce prodigious amounts of reaction without being harmed. For instance, it has been stated that *invertase* will invert 1,000,000 times its own weight of sugar and not be harmed.

4. *Enzyme reactions are reversible.*—Most enzymes are known on account of their ability to decompose complex substances. However, when some enzymes are put in contact with the products which they usually form, they synthesize the substance which they usually split. If maltase is put into dextrose and other proper conditions maintained it causes the formation of maltose. When lactase is put into a mixture of galactose and dextrose, lactose is formed. Euler suggested that we indicate the synthetic ability of an enzyme by using the suffix *ese* in place of *ase*, which signifies decomposition. It should be emphasized before leaving this subject that the amount of compound built up by the synthetic action of an enzyme is insignificant in comparison with the amount split up.

5. *Enzymes stimulate formation of anti-enzymes.*—When enzymes are injected into the blood stream of an animal they tend to cause the formation of anti-enzymes. These are bodies which neutralize or prevent the action of the enzyme.

6. *Possess some properties of living cells.*—It is interesting to compare enzymes with living cells. It is seen that they possess many characteristics in common.

Living Cells	Enzymes
1. Temperature	1. Temperature
a. Destroyed by high temperatures	a. Inactivated by high temperatures
b. Have optimum temperature for action	b. Also have optimum temperature for action
c. Action slowed up at low temperatures	c. Action slowed up at low temperatures
2. Require certain salts	2. Certain salts necessary
3. Require certain limits of reaction (pH)	3. Require certain limits of reaction (pH)

7. *Enzymes are sensitive to the presence of acids, alkalis and salts in their environment.*—Some enzymes require the presence of a certain hydrogen-ion concentration. For instance, peptase (pepsin) in the stomach requires 0.2 per cent of hydrochloric acid but, like most enzymes is inhibited and destroyed by large amounts of acid.

8. *Enzymes may require the presence of certain materials called Coenzymes.*—These are substances which are necessary for the action of the enzymes. They are best illustrated by the phosphates which have been found to be necessary in the functioning of *zymase* in alcoholic fermentation.

9. *Some enzymes must be liberated by a substance called a Proenzyme or Zymogen.*—The proenzyme is inactive and must be activated in some manner. It is not as well established for bacterial enzymes as for certain of the digestive enzymes.

10. *Enzymes are colloidal in nature, usually, and are therefore generally non-dialyzable.*—This is a general statement on which more information is desirable.

Systematic Arrangement or Classification of Enzymes.—The diversity of types and groups of enzymes allows several different ways of grouping. They may be divided on the basis of whether they do their work inside or outside of the cell, on the basis of type of chemical change produced, or on the basis of type of compound decomposed. Several systematic arrangements have been published, all of which have merits.

We will use Buchanan's classification of enzymes because it is especially valuable for those working with single-celled microorganisms. Other classifications may be found in Waksman and Davison's "Enzymes."

CLASSIFICATION OF ENZYMES

(After Buchanan, 1924)

1. Basis of localization of enzyme.
 - a. Intracellular
 - b. Extracellular
2. Basis of preponderance of action being.
 - a. Analytic
 - b. Synthetic
3. Basis of type of action.
4. Basis of substance attacked.
5. Basis of substance produced.

Classification of Enzymes of Microorganisms

- a. Hydrolytic and dehydrating enzymes. *Hydrolases*.
- b. Action primarily hydrolytic. Catalyzing the hydrolysis of
 - c. Fats and esters. *Lipases* and *esterases*.
 - 2c. Carbohydrates. *Carbohydrases*.
 - d. Polysaccharids.
 - e. Cellulose. *Cellulase* or *Cytase*.
 - 2e. Pentosans.
 - 3e. Pectins. *Pectinase*.
 - 4e. Agar-agar.
 - 5e. Gums, etc.
 - 6e. Starch. *Amylase* or *diastase*.
 - 7e. Inulin. *Inulase*.
 - 2d. Tetrasaccharids.
 - e. Stachyose. *Stachyase*.
 - 2e. Lupeose. *Lupease*.
 - 3d. Trisaccharids.
 - e. Raffinose. *Raffinase*.
 - 2e. Melezitose. *Melezitase*.
 - 3e. Gentianose. *Gentianase*.
 - 4e. Verbascose.
 - 5e. Secalose.
 - 6e. Mannatrisaccharids.
 - 4d. Disaccharids.
 - e. Hydrolyzing to hexoses only.
 - 2f. Hydrolyzing to 2 molecules of glucose.
 - g. Hydrolyzing sugars which reduce.
 - Fehling's solution.
 - h. Maltose. *Maltase*.
 - 2h. Iso-maltose.
 - 3h. Cellobiose.
 - 4h. Gentiobiose.
 - 5h. Turanose.
 - 2g. Hydrolyzing non-reducing sugars.
 - h. Trehalose. *Trehalase*.
 - 2h. Isothelalose.

- 2f. Hydrolyzing to glucose and galatose.
 - 2g. Melibiose. *Melibiose*.
 - 3f. Hydrolyzing to glucose and fructose.
 - g. Sucrose. *Sucrase* or *Saccharase*.
 - 2e. Hydrolyzing to pentose and hexose.
 - f. Vicianose. *Vicianase*.
 - 5d. Glucosides.
 - e. Amygdalin. *Emulsin*.
 - 2e. Tannin. *Tannase*.
 - 3e. Phytin. *Phytase*.
 - 3c. Proteins and their split products.
 - d. Producing coagulation—*Coagulases*.
 - e. Casein. *Rennet*—*lab*—*chymosin*.
 - 2e. Fibrinogen. *Thrombase*.
 - 2d. Action not primarily a coagulation.
 - e. Hydrolyzing proteins. *Proteases*.
 - f. Gelatin. *Gelatinase*.
 - 2f. Casein. *Casease*.
 - 3f. Protein. *Erepsin*.
 - 4f. Protein. *Trypsin*.
 - 5f. Protein. *Pepsin*.
 - 2e. Hydrolyzing nucleic acid. *Nucleases*.
 - 3e. *Amidases* and *desamidases*.
 - f. Urea. *Urease*.
 - 2f. Adenin. *Adenase*.
 - 3f. Guanin. *Guanase*.
 - 4f. Amino acids. *Desamidase*.
 - 5f. Uric acid.
 - 6f. Hippuric acid.
- 4e. Lysins (probably largely proteases and nucleases).
 - f. Bacterial cells, etc. *Pyocyanase*.
- 2f. Red blood cells. *Hemolysins*.
- 3f. Bacteria. *Bacteriolysins*.
- 4f. Bacteria. *Bacteriophage*?
- 2b. Action primarily dehydrating and synthetic.
- 2a. Oxidizing and reducing enzymes. *Oxido-reductases*.
 - b. Catalyzing oxidation by use of free oxygen or reduction with production of free oxygen.
 - c. Action primarily oxidative.
 - d. Respiratory enzymes.
 - 2d. Photogenic enzymes. *Luciferase*.
 - 3d. Enzymes described as oxidizing specific substances.
 - e. Tyrosin. *Tyrosinase*.
 - 2c. Chinic acid.
 - 3e. Alcohol (to acetic acid). *Alcoholase*.
 - 4e. Alcohols and polyatomic atomic alcohols to sugars and acids.
 - 5e. *Acid oxidases*.
- 2c. Action primarily reductive.
 - d. Peroxides.

- 2b. Catalyzing oxidation or reduction without use or production of free oxygen.
- c. Catalyzing oxidation of one compound with simultaneous reduction of another.
 - d. Reducing peroxides and oxidizing other compounds. *Peroxidases*.
 - 2d. Reducing organic pigments as litmus, methylene blue.
 - 3d. Reduction of inorganic compounds, as nitrates, sulphates, etc. *Philothion* of yeast.
 - 2c. Catalyzing simultaneous intramolecular oxidations and reductions.
 - d. Sugar to alcohol and CO_2 . *Zymase*.
 - 2d. Sugar to lactic acid. *Lactolase*.
 - 3d. Glycolyse (?)
 - 4d. Glucose. (?)
 - 5d. Decomposing hydroxy and keto acids to alcohols or aldehydes and CO_2 . *Carboxylase*.

Hydrolytic Enzymes.—These are also spoken of as hydrolases and are widely formed in nature. As their names indicate, they bring about the hydrolysis of complex substances by means of water. It is impossible, as well as unnecessary, to discuss all of the enzymes under each heading. Only a few of the more common ones will be discussed here.

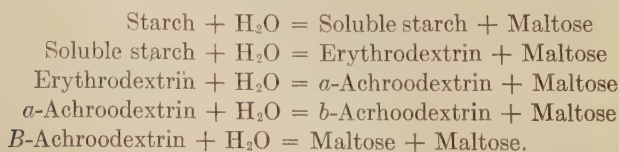
Cellulase.—By means of this enzyme, bacteria are able to hydrolyze cellulose. It is present in some of the bacteria causing plant diseases, especially those diseases which are accompanied by a great amount of destruction of tissue. The chemical constitution of cellulose is not definitely known but since it is a complex carbohydrate the equation may be written.



The product in this case is called cellobiose.

Pectinase.—This enzyme hydrolyzes the complex polysaccharide pectose. Pectase-secreting organisms are probably of importance in the retting of flax. The cellulose fibers in the flax plant are surrounded by a pectose binder. In order to liberate these fibers, the flax stalk is subjected to anaerobic fermentation. This enzyme is also formed by many of the bacteria causing plant diseases.

Amylase or Diastase.—This is the starch hydrolyzing enzyme. Starch is progressively decomposed through a number of stages. These have been given by Mathews as follows:



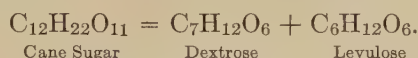
These several products of hydrolysis of starch have different colors with

iodin and explain the varying shades of blue-lavender and red which are often seen when the hyrolysis of starch is followed with iodin. The bacteriologist uses the ability to decompose starch as one of the differential characteristics of pure cultures.

Amylase is present in *malt* which was often added to various materials on account of its starch hydrolyzing ability. Malt is prepared by allowing barley to germinate for a time after which germination is stopped by heating. This gives a material which is rich in amylase.

Amylase is also formed by many of the molds. In the making of industrial alcohol by the amylo process a mold forming very active amylase hydrolyzes the starch to fermentable sugars.

Invertase.—This is the enzyme which causes the inversion of cane sugar or its decomposition into dextrose and levulose.



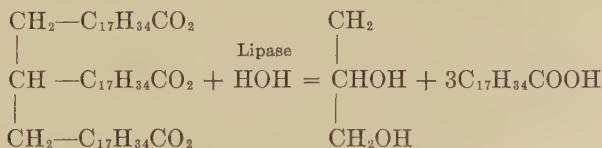
It is easily prepared from beer yeast by putting the cells in chloroform to inhibit budding and growth, and heating to 30° C. After a short time the mixture is filtered and the filtrate tested for its inverting ability. The enzyme is quite widely distributed through the world of microorganisms. The name *invertase* is used synonymously with *sucrase*.

Maltase.—This enzyme hydrolyzes the disaccharide maltose to two molecules of glucose. The following formula will represent this change.



The enzyme is widely distributed in nature in both animal and plant cells.

Lipase.—This enzyme was formerly known as steapsin or lipolytic enzyme. The latter name is probably not a good one since it implies a decomposition by means of fat. Fats are known as triglycerides by the chemist or as combinations of glycerol with fatty acids. Consequently, when fats are hydrolyzed by lipase they react with three molecules of water to form glycerol and fatty acids. This enzyme is formed by a number of microorganisms.



Enzymes which decompose esters are called esterases.

Peptase, Tryptase and Ereptase (Proteases).—These three protein-splitting enzymes may be discussed together since they are similar in

function. They are the enzymes which are important in protein decompositions in the animal alimentary tract. They do not bring about the same type of decomposition.

Peptase.—This enzyme is present in the gastric juice of animals. It acts upon native proteins and demands a medium which is slightly acid. It changes proteins to proteoses and peptones.

Tryptase.—This enzyme hydrolyzes proteins to amino acids and acts best in an alkaline solution. It is secreted into the intestines.

Ereptase hydrolyzes the peptones and some of the polypeptids to amino acids. It cannot hydrolyze the proteins. It is an intestinal enzyme.

Rennin.—This enzyme is the coagulating enzyme of the stomach. It is, perhaps, less known under the names *lab* and *chymosin*. It is prepared from the stomachs of swine and after purification and concentration is used in cheese making. The casein in the milk is changed into paracasein. The latter is a product of hydrolysis and therefore has a smaller molecule. This is indicated by simple experiments in every course in biochemistry. The addition of alkali to an acid curd will restore the casein to its original condition but with a rennin curd this is impossible.

Zymases.—The term zymases used in this discussion includes all of the intracellular enzymes regardless of whether they attack carbohydrates or proteins. The term is often restricted to the enzyme which functions in alcoholic fermentation. We shall use it in the first manner as including all of the endoenzymes. The term may be made more specific, as Giltner has done, by qualifying it with a prefix giving dextrozymase, galactozymase, etc., depending on the substrate decomposed.

We may discuss a few of the more important zymases or endoenzymes of the cell. One of the most important is that contained in so-called yeast juice—the juice pressed from the yeast cell according to the procedure outlined earlier in this chapter. This is frequently called Buchner's zymase. Harden and Young found that when Buchner's yeast juice was dialyzed, it was composed of two parts, a *residue* which did not dialyze and a *liquid* which did. The residue was inactive as was the liquid. Both had to be present in order to secure fermentation. Consequently, the liquid was called a *coferment* or *coenzyme*. Further experimental work indicated that the *coferment* was heat resistant. This suggests that enzymes are more complex in constitution than admitted by many.

Oxidizing Enzymes (Oxidases).—Not as many oxidases are known although an oxidizing enzyme may be created for any oxidation brought about by bacteria. Oxidizing enzymes function in the making of vin-

egar. *Alcoholase* and an aldehyde-oxidizing enzyme, *aldehydase*, are concerned. Alcoholase would oxidize the ethyl alcohol to acetaldehyde and the acetaldehydase the acetaldehyde to acetic acid.

Reducing Enzymes (Reductases).—Reductases, as the name indicates, reduce compounds by taking away oxygen. This action is used in some of the most important tests in food control and sanitary work. The best-known reductase is catalase which decomposes hydrogen peroxide into water and oxygen. Besides this reductase, there are those which reduce the dyes to colorless leuco-bases. Such a change is noticeable in litmus milk tube where the pink color of the litmus is changed to white at the bottom of the tube. It is also demonstrable with methylene blue.

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CHAPTER XVI

NITROGEN METABOLISM (CYCLE); SULFUR METABOLISM (CYCLE)

Occurrence of Nitrogen.—About 80 per cent of the atmosphere is nitrogen. It is also found dissolved in water. Such nitrogen is spoken of as *free nitrogen* in contrast with *fixed nitrogen*; the latter is in the form of salts such as nitrates, nitrites, ammonia compounds, etc. Large deposits of fixed nitrogen are not abundant. The greatest and best-known deposits are in Chile where deposits of guano contain vast amounts of nitrates. Slosson stated that, since their discovery in 1809, 53,000,000 tons of sodium nitrate, "saltpeter," have been removed. Predictions are that this deposit is giving out and that we must accordingly look elsewhere for large amounts of nitrogen. It is no wonder that scientists turned to the atmosphere to attempt fixing this incomprehensible supply. In this book we need not be concerned with all of the methods which man has devised for taking the nitrogen out of the atmosphere but with only those methods which are microbiological in nature.

Above each acre of land are about 75,000,000 pounds of nitrogen, a supply which is always available and which should be utilized.

In discussing the nitrogen cycle, our attention goes to the soil and its nitrogen content. Hopkins stated that the brown silt loam of Champaign County, Illinois, contained only 4760 pounds of nitrogen per acre; a hundred-bushel corn crop for the grain alone requires 100 pounds of nitrogen. The nitrogen supply, then, is sufficient for only forty-six years. On account of this there must be some method of replenishing the nitrogen. Modern agriculture has one of its greatest problems here. The greater portion of nitrogen in the soil is in the form of organic matter. This residual stock is altered in many different ways. Some of it is removed in the crop, some is washed away by drainage water and some goes into the atmosphere by bacterial changes which are discussed below.

Significance of Nitrogen to Life.—Nitrogen is a very important element for living organisms. It is required by all forms of life for growth and repair. Nitrogenous compounds are present in all kinds

of living tissue. According to Lotka, the proportion of nitrogen to carbon in the human body is 1 : 3 and in the atmosphere 5500 : 1.

The Nitrogen Cycle.—As stated above under the discussion of nutrition of bacteria, cycles are used to show the relationship of the various stages through which compounds of elements such as nitrogen are made to pass by various agencies. Many cycles have been prepared; all of them are founded on the same facts. The one shown here is adapted from one given by Fowler in his "Bacteriological and Enzyme Chemistry." Examination of this cycle will indicate that nitrogen may follow one of two paths. It may remain in the fixed condition on the surface of the earth or it may travel a longer circuit or cycle and pass into the air, to

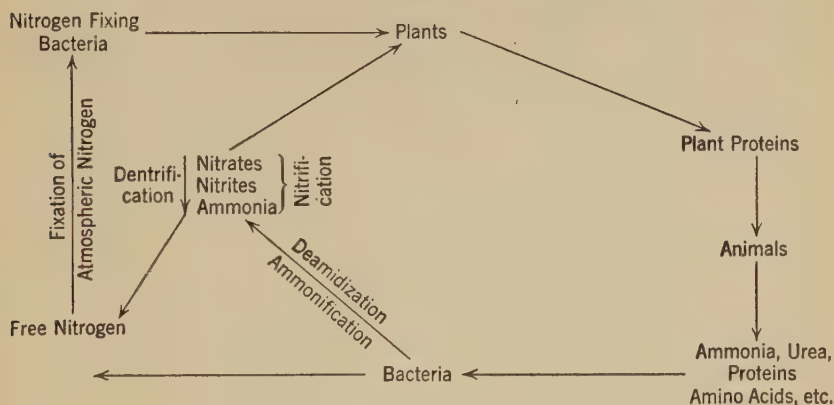


FIG. 99.—Nitrogen Cycle. (Adapted from Fowler.)

return later to the earth by any of the methods which are available for fixation. No single group of living organisms is more concerned with forcing nitrogen along its path than any other. Every microorganism plays a rôle. The bacteria are, perhaps, more active in taking nitrogen from the atmosphere than other organisms.

Fixation of Atmospheric Nitrogen.—This is a fitting place at which to begin our discussion of the nitrogen cycle since it starts with that tremendous supply of nitrogen in the atmosphere. The term fixation in this case means the changing of the free nitrogen of the atmosphere to the fixed state. It means, then, the removal of nitrogen from the atmosphere and its fixation on the earth where it becomes subject to man. The World War greatly stimulated interest in the various possible methods of nitrogen fixation. Warring nations found it necessary to have an abundant supply of the element at low cost without the hazards of shipping it from country to country. Consequently, they turned to the

atmosphere and perfected methods of chemical fixation. Since the War a few attempts have been made to convert some of the war-time plants to the production of nitrogenous compounds for the farmer.

Outline of Methods of Nitrogen Fixation.—It is best to take a broad view of the whole subject of nitrogen fixation and, at least, mention the more important methods. These are grouped under two main headings, the chemical methods and the biological methods. The chemical methods are mentioned only because they supplement the biological methods as well as for completeness.

I. Chemical Methods of Fixation.

- A. Burning phosphorus in confined air.
- B. Distilling liquid air.
- C. Electrolytic fixation.
- D. Fixation by silent electric discharge.
- E. Fixation by Haber process (direct union of N and H under pressure).

II. Biological fixation.

- A. By fungi.
- B. By bacteria.
 1. Non-symbiotic
 - a. Aerobic method
 1. Azotobacter
 - b. Anaerobic method
 1. Clostridium pasteurianum, etc.
 2. Symbiotic fixation
 - a. Rhizobium leguminosarum

CHEMICAL METHODS OF NITROGEN FIXATION

One method is the distillation of liquid air. Air is liquefied at a very low temperature. As the temperature is raised the various constituents come off and are collected. Another method is the burning of phosphorus in confined air. The phosphorus combines with the oxygen leaving the nitrogen. Oxygen may also be removed by passing the air over red-hot copper in a confined space. Copper oxide (CuO) is thus formed. The Haber process of nitrogen fixation is widely used and has, perhaps, the greatest possibilities. In this process ammonia is made by the direct union of nitrogen and hydrogen under pressures up to 200 atmospheres. Whiting and Fred stated that, in 1923, fully 36 per cent of the nitrogenous compounds used in chemical and fertilizer industries were obtained in the several chemical methods of fixation. The remainder came from natural deposits. These methods, however, are expensive and the resulting compound is expensive.

BIOLOGICAL METHODS OF NITROGEN FIXATION

These are the methods with which we are especially concerned. They are separated from the chemical methods by the fact that some living cell is concerned with the process. Several different types of cells have been found to fix nitrogen, quite important among which are the bacteria.

Non-symbiotic (Asymbiotic) Fixation of Atmospheric Nitrogen.—

This method differs from the symbiotic method in that there is no growing together of organisms in partnership and harmony. In 1885 Berthelot reported that nitrogen increased in soils that were not being cultivated. Later on, Winogradsky, isolated a number of bacteria which could take nitrogen out of the atmosphere and fix it for plants' use. Still later, Beijerinck studied the problem and made distinct additions to our knowledge.

Aerobic Non-symbiotic Fixation.—Winogradsky and Beijerinck both isolated aerobic bacteria which could fix nitrogen alone. Beijerinck isolated and described a large aerobic organism, to which he gave the generic *Azotobacter*. Beijerinck was under the impression that the *Azotobacter* had to have some other bacteria present. This was finally disproven and it was demonstrated that they could fix nitrogen alone in pure culture. *Azotobacter chroococcum*, *Azotobacter agilis*, *Azotobacter vinelandii* and *Azotobacter Beijerinckii* are representative members of the genus. This method of nitrogen fixation is called Azofication. The term is not a good one since from the Latin constituents it means the making of nitrogen and not the fixation of nitrogen.

The energy relations of these organisms are especially interesting. The fixing of nitrogen is an endothermic reaction. The bacteria have to secure energy from other compounds. Since the bacteria have no chlorophyll, they cannot use the energy in sunlight and, as we have learned before, must resort to the chemical decomposition of large organic molecules. The carbohydrates or sugars are the best energy-yielding compounds. Numerous students have reported the amount of nitrogen fixed per unit of carbohydrate used up. *Azotobacter vinelandii* has been reported to fix between 15 and 20 mg. of nitrogen for every gram of mannite used for energy. The amount fixed, of course, varies greatly with the species and other conditions.

Anaerobic Non-symbiotic Nitrogen Fixation.—Certain anaerobic bacteria can also take nitrogen from the atmosphere. *Clostridium pastorianum* is a member of this group of organisms. These forms are widely distributed in soil and will grow under anaerobic conditions in the presence of carbohydrates and certain mineral matter.

The energy relationships of these organisms are also interesting. The anaerobic nitrogen fixers are not as efficient as the aerobic *Azotobacter*. Winogradsky reported that *Clostridium pastorianum* would fix from 2 to 3 mg. of nitrogen for each gram of sugar used for energy.

Fixation of Nitrogen by Higher Plants and Fungi.—The significance of some of the higher plants such as yeasts and molds in nitrogen fixation has also been studied. While these organisms were found to fix some nitrogen the amount was so small in comparison with the other methods



FIG. 100.—Red Clover; Effect of Nitrogen-fixing Bacteria. No Nitrogen in the Soil of Either Pot. (After Hopkins, 1904.)

that it is insignificant. It is also quite possible that the technic used with these other organisms is not above question.

Symbiotic Fixation of Atmospheric Nitrogen.—Certain of the *Leguminosae* have been used for centuries as soil improvers. It was known that they did something to the soil which made it more suitable for the crops which followed. The Roman farmers observed that beans enriched the soil; they plowed lupines under to further the enrichment. The history of this work need not concern us to any great extent; its importance, however, justifies a little more space than some other subjects. Thought and study were given to the solution of the question of just how the legumes were able to enrich the nitrogen supply in the

soil. Finally it was revealed that the nodules on the roots of these plants had something to do with this process. Woronin (1866) found that bacteria were present in them and also explained them as pathological processes. Later on it was found that sterilization of the soil inhibited the formation of nodules, thus indicating that they were, perhaps, biological in origin. A distinct advance in knowledge was made by Ward, the great English botanist. He found that he could inoculate the roots of young legumes by placing them in contact with old nodules.



FIG. 101.—Showing the Effect of Inoculation of Legumes with Nitrogen-fixing Bacteria. Vines from a Field where Peas Had Not Been Grown Before. (After Whiting, Fred, and Stevens.)

We are told that in Germany soy beans could not be grown until some of the soil from their natural habitat had allowed inoculation. It was soon evident that the cause of nodule formation came from outside of the plant. The discovery of the symbiosis between the bacterium and the plant is probably due to the work of Hellriegel and Wilfarth. They found that sterilization of the soil caused legumes to grow as non-legumes and eventually to die of starvation. Thus we learned of this great provision that nature had made to maintain a supply of nitrogen in the soil. Since these reports, an endless line of experiment has been continued to find out more about this relationship.

The amount of nitrogen fixed by bacteria is enormous. Whiting and Fred stated that if 10 per cent of the cultivated land in the United States had been in leguminous crops in 1924, and the average fixation had been 50 pounds per acre, 925,000 tons of nitrogen would have been

taken from the air. This indicates that the growing of leguminous crops is indeed a cheaper source of nitrogen than are the commercial fertilizers.

The Nitrogen-fixing Bacterium.—The discovery of the bacteria in the nodules or tubercles, as the little projections on the roots of legumes are called, was slow and beset with interesting discussion. Beijerinck, the Dutch bacteriologist, was probably the first to secure a pure culture of the nitrogen-fixing bacterium. With pure cultures he was able to study the characteristics of the organism. He gave it the name *Bacillus radicumicola*. Another investigator by the name of Frank, a German, studied some bacteria from nodules which he believed were the true nodule nitrogen-fixing bacteria. He did not use the name proposed by Beijerinck but introduced another, *Rhizobium leguminosarum*. Since these names were proposed, considerable controversy has centered about the question of which is the proper name. It is quite definitely known that Frank probably did not have the right organism. However, he gave the name *Rhizobium leguminosarum* to the organism which fixes nitrogen symbiotically. This name has priority over the name *Bacillus radicumicola* and, therefore, is the one which should be used.

Nodule (Tubercle) Formation.—When the proper bacteria are available, nodules or tubercles appear on the roots of the plants. In them are found the bacteria which take the nitrogen from the atmosphere. These appear early on the roots and are very small at first. About a month is required before they can be seen without great difficulty.

Relation of Organisms to the Plant.—This is not an easy question to settle. Since we have here two different organisms living in very close proximity it is not surprising that about all the explanations that could be advanced were offered to explain the relationship. We may review some of them.

Parasitism.—Some suggested that this was a case of parasitism, the legume being the host and the bacterium the parasite. This would imply some competition between the two organisms. What evidence is there for this? In the strict sense this might be regarded as parasitism since we have one organism living on another, but in most cases of parasitism the parasite gives nothing in return. Some have called it a case of parasitism which slowly turns into a mutual symbiosis.

Mutual Symbiosis.—This explanation is just the opposite of the one just discussed. According to this, the relation of these two organisms is a partnership agreeable to both forms. Whiting has defined a mutual symbiosis as the “contiguous association of two or more morphologically distinct organisms not of the same kind, resulting in an acquisition of assimilated food substances. It implies that the organisms concerned have the power of independent existence, but that both are benefited

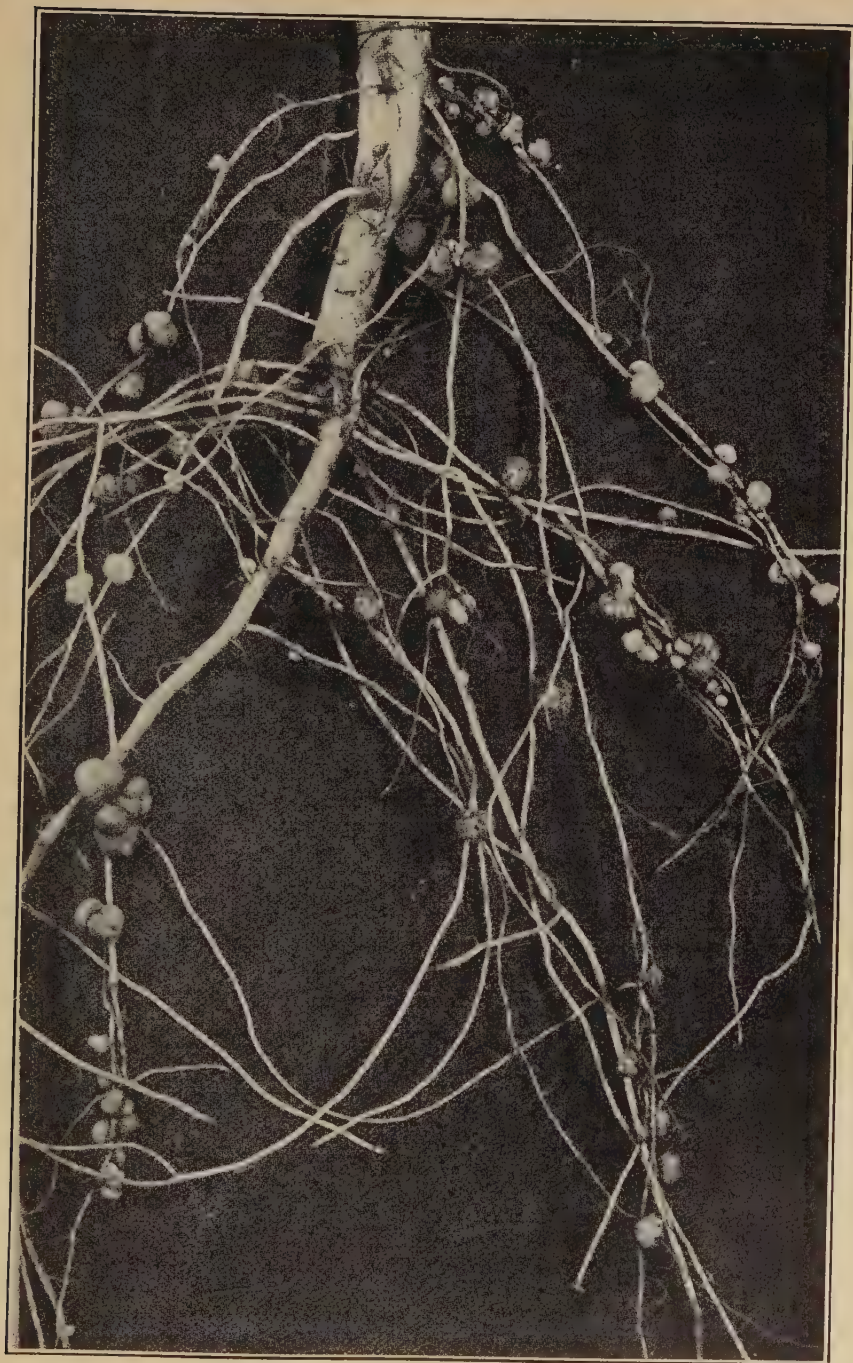


FIG. 102.—Showing Cowpea Root Tubercles. Natural Size. (After Hopkins, 1904.)

by the close association." We may reasonably ask how one organism benefits the other in this case. The mere living together would hardly explain it. It is explained by the fact that the bacterium secures carbohydrates from the plant and in return gives the plant the nitrogen which is so necessary for its growth and repair. In contrast with non-symbiotic methods of nitrogen fixation, the symbiotic method is a constructive one since organic matter is bound to increase in the soil.

Soil Inoculation Methods.—Since it has been shown that the symbiotic fixation of atmospheric nitrogen is desirable and since certain soils are poor in bacteria which work with legumes to fix it, methods are used for getting these bacteria either onto the land or onto the seeds. These are known as inoculation methods.

Soil Transfer Method.—Fields which have not grown the legume which it is desired to raise, may be inoculated with the desirable bacteria by taking some soil (leguminous earth) from a field which has grown the desired legume, and spreading it over the new field. When the seed is sown on such a field, it germinates and the young plant soon encounters the bacteria. This method is less direct than the culture method where the bacteria are applied directly to the seed.¹ It may also be inconvenient to transfer a sufficient amount of earth from an old field to a new one.

Culture Method.—The bacteria, which are isolated from the nodules on the roots of legumes may be grown in pure culture on laboratory media and distributed to the farmer in this way. Such cultures when prepared by reliable laboratories possess distinct advantages when applied directly to the seed. When the bacteria are applied to the seed they are ready to inoculate the rootlets of the young plants during the early stages of growth and thus to make atmospheric nitrogen available. This gives the plant a start which it might not enjoy if natural inoculation were relied upon, for the rootlets would have to wait until they had come in contact with the bacteria in the soil. This results in delayed growth. One of the older methods for getting the bacteria onto the seed is the so-called glue method devised at the Illinois Agricultural Experiment Station. About two handfuls of glue are dissolved in a gallon of water. After cooling, this is sprinkled over the seed at the rate of one pint per bushel. After stirring, the leguminous earth in dry powder

¹ As soon as it had become firmly established that inoculation of such seed caused increases in crop yields, a number of commercial preparations appeared on the market. Many of them were found later to be worthless since they would not bring about the formation of nodules on legumes, the ultimate test for a bacterium which is advertised as fixing nitrogen symbiotically. The situation, in this case, is not greatly different from that in other cases such as chemical disinfection, so-called patent medicines, etc. Some states have been driven to legislation for controlling such preparations.

form is sprinkled over the seed and sticks to the seed. More recent practice seems to indicate that the glue is not necessary. The bacteria adhere to the seed sufficiently well.

It is apparent that to secure good inoculation the proper bacterium should be used and that the culture should be strong and healthy. Inoculation methods received some criticism because in the past agriculturists were exploited by being given poor cultures. Attempts at inoculation were unsuccessful and on account of this the whole system was regarded as questionable. There are, to-day, reliable laboratories that produce good cultures.

Are there Different Species of Legume Bacteria?—Evidence points to the conclusion that there are different varieties of legume bacteria. The planting of certain legumes on a field that has not grown them, or certain closely related forms may not result in inoculation and good nodule formation. Soil bacteriologists have divided the legumes into groups which will cross-inoculate. Some of these groups are:

<i>Group 1</i>	<i>Group 3</i>	<i>Group 5</i>
Red clover	Cowpea	Soy bean
Mammoth clover	Lima bean	
Crimson clover	Peanut	
Alsike clover	Lespedeza	<i>Group 6</i>
White clover	Velvet bean	Navy bean
	Kidney bean	Kidney bean
		String bean
	<i>Group 4</i>	Wax bean
	Garden pea	
<i>Group 2</i>	Field pea	
Alfalfa	Hairy vetch	
Sweet clover	Spring vetch	<i>Group 7</i>
Hubam	Sweet pea	Dalea (Wood's clover)

It will be noticed that the sweet clover and alfalfa bacteria are in the same group, and will cross-inoculate. These bacteria are probably quite widespread in nature by the growth of wild sweet clover along highways and railroads. The bacteria-inoculating plants in the other groups are not so common and the agriculturist may have to resort to artificial inoculation to secure good nodule formation.

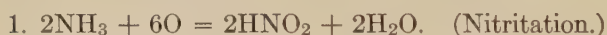
How is the Nitrogen Transferred to the Plant?—Different explanations have been offered. Perhaps none of these is correct or perhaps they are all correct to a certain extent. One explanation suggests that the bacterial cells are absorbed by the plant. In this manner the organic nitrogenous matter of the bacterial cells is used by the plant. Another

explanation is that the bacteria grow in the plant and excrete nitrogenous matter which is taken by the plant. Biochemists have not been successful in isolating the nitrogenous substance taken from the bacterium by the plant. Various other explanations, some of them quite fantastic, have been offered. We need not consider them but leave them for later study.

NITRIFICATION

This term signifies the making of nitrates by oxidation from ammonia and nitrites. For a long time the method of oxidation was unknown. Some said that it was chemical. The fact that nitrification is a biological process was shown by Schloesing and Müntz. They carried out an experiment in which there was oxidation of ammonia to nitrates. The ammonia disappeared while nitrates increased. Suspecting that it was a process, they added chloroform to another experiment and secured no oxidation. The chloroform stopped nitrification. This proved that it was a biological process even though they could not isolate a specific organism which brought about the oxidation.

Isolation of Nitrifying Bacteria.—Bacteriological research soon revealed that the oxidation of nitrogen in reduced compounds, such as ammonia, took place in two stages.



The organisms were called *Nitrosomonas* and *Nitrosococcus* for nitritation and *Nitrobacter* for nitrataion. Winogradsky started the experiments which resulted in the discovery of these organisms. They were found to be extremely sensitive to the presence of organic matter. Consequently the ordinary media which were being used at the time, and which contained much organic matter, did not reveal these organisms. Finally by resorting to the use of inorganic media, Winogradsky was able to isolate an oval organism which oxidized ammonia.

DENITRIFICATION

This is the opposite of nitrification. Since nitrification is an oxidation process, denitrification is a reduction process. This reduction may be brought about in either of two ways. It may be accomplished by bacteria which possess *reductases*, reducing enzymes, or it may be accomplished by chemical reducing agents formed by bacterial action on organic matter. Reductions which are brought about by bacterial enzymes usually result in the formation of free nitrogen while the chemical reduc-

tion results in any of the various products representing intermediate stages in the oxidation of nitrogen.

The terms nitrate reduction and denitrification are sometimes used synonymously. The latter term, denitrification, should probably be restricted to the decomposition of nitrates and nitrites to free nitrogen. Nitrate reduction would involve simply the reduction of nitrate to nitrite.

The property of denitrification is quite widespread among bacteria. The products of reduction are also varied. In 1886, Gayon and Dupetit isolated an organism which they called *Bacillus denitrificans*; this organism reduced nitrates with the formation of gaseous nitrogen. Other varieties of this species have since been isolated.

A number of other common bacteria have been shown to reduce nitrates but many of them stop with ammonia rather than free nitrogen. Besides these common bacteria, certain molds and yeasts also reduce nitrates. This property then seems to be quite widespread among the various groups of microorganisms.

AMMONIFICATION

This term covers the formation of ammonia from various compounds by microorganisms. The subject may be divided under two subheadings: the formation of ammonia from inorganic compounds and its formation from organic compounds.

Ammonia Formation from Inorganic Compounds.—The formation of ammonia from inorganic compounds is best accomplished from those which are in a higher state of oxidation. This would then involve reduction, which has been discussed under denitrification.

Ammonification from Organic Compounds.—Ammonia formation from organic compounds is best explained by the deaminization of the amino acids. This process takes off the NH_2 group, leaving a fatty acid. This deaminization is the first step in the decomposition of the amino acid.

Ammonifying Bacteria.—Many of the common bacterial types are ammonifiers. The members of *Bacillus subtilis* group are especially active in this respect.

DECOMPOSITION OF PROTEINS

PUTREFACTION

The term putrefaction is generally restricted to the decomposition of proteins and the split products of proteins. It is usually distinguished from fermentation, which in this instance may be looked upon as the decomposition of carbohydrates. This distinction is a perfectly arbitrary.

trary one and has probably come down to us from early times in the science.

Significance of the Nitrogen Cycle.—Most of the discussion in the preceding paragraphs has been based upon the relation of the nitrogen cycle to agriculture. It is just as useful and convenient in other lines of applied bacteriology. The sanitary chemist uses the different stages in the oxidation of nitrogen as criteria of the sanitary quality of water. These applications will be discussed later on. The treatment of sewage also depends on attempts to change the state of oxidation from the reduced to the completely oxidized stage. Consequently, the nitrogen cycle has an important place in applied biology.

Putrefaction or Anaerobic Decomposition of Proteins.—Most typical putrefactions are anaerobic. The products are left in an unstable reduced condition. Such products usually have bad odors and cause the nuisances resulting from putrefactions. They include indole, mercaptans, hydrogen sulfide, etc. The products of anaerobic decomposition of proteins are usually incompletely oxidized since they are formed under conditions where no oxygen is available for oxidation. The putrefactive bacteria are many. Among the more common ones are *Clostridium putrificum*, the members of the proteus group, *Escherichia coli* (*Bacterium coli*), etc. The last-mentioned organism is an inhabitant of the human intestines. The criteria of typical putrefaction have been expressed as follows by Phelps:

1. Development of offensive odors.
2. Formation of a black sediment or residue.
3. Reduction in the amount of dissolved or free oxygen.
4. Reduction in the amount of available oxygen.
5. Increase in the carbonaceous (oxidizable) matter.

Putrefaction and fermentation have some general characteristics in common. Both involve the decomposition of large molecules and both yield products which are useful to the cell. Both yield energy but the amount is much greater, of course, from fermentation of carbohydrates.

Decay or Aerobic Decomposition of Proteins.—Decay is a term which has been used to signify the aerobic decomposition of proteins. It differs from putrefaction mainly by the state of oxidation of the products. Since decay is an aerobic process, the products are completely oxidized to stable conditions. Where we have hydrogen sulfide (H_2S) as a product in putrefaction, we would have the sulfur appearing as SO_4 in decay.

It is important to point out, without reviewing the experiments, that bacteria are unable to decompose pure native proteins. By this

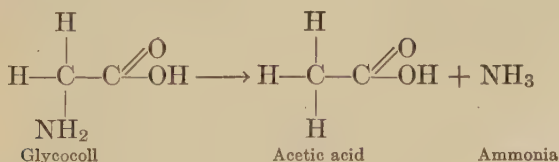
is meant pure washed proteins such as edestin, zein, etc., which have been prepared by the most careful technic and added to pure inorganic media. It seems necessary that simpler substances be present for the initial energy and growth requirements of the microorganisms. When growth has started, sufficient enzymes are secreted with which the protein molecules are decomposed. Experimental evidence is also available to show that the peptones and proteoses are just as resistant as are the proteins.

Decomposition of Protein Split Products.—The proteins are such complex compounds that a great number of so-called split-products, or decomposition products, are possible. The proteoses and peptones may be broken into amino acids and a host of other compounds. The protein molecule breaks down through the following stages: proteins, proteoses, peptones, polypeptids and amino acids. The destruction of a number of these compounds in bacterial metabolism has been discussed in a number of different places in this book. Since certain products resulting from the putrefaction of amino acids are used by bacteriologists for following bacterial changes in media, it is proper, perhaps, that their origin be explained. We need not be concerned with the products that result from the decomposition of all of the amino acids but just those of special interest to bacteriologists; such are tryosine and tryptophane.

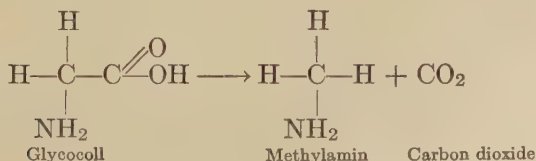
Before discussing the decomposition of specific amino acids, however, it might be proper to give some of the general reactions by which all amino acids are decomposed.

The chemistry of the putrefactive changes which take place in the intestines is fairly well known. Straight chain acids are among the first compounds which are attacked and the changes may be indicated as follows:

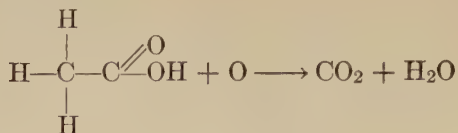
I. Deaminization.



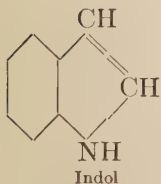
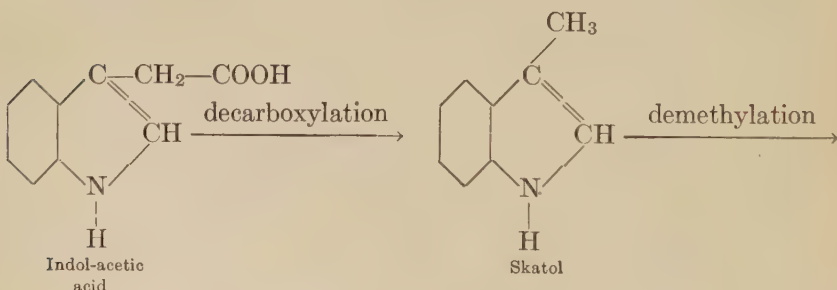
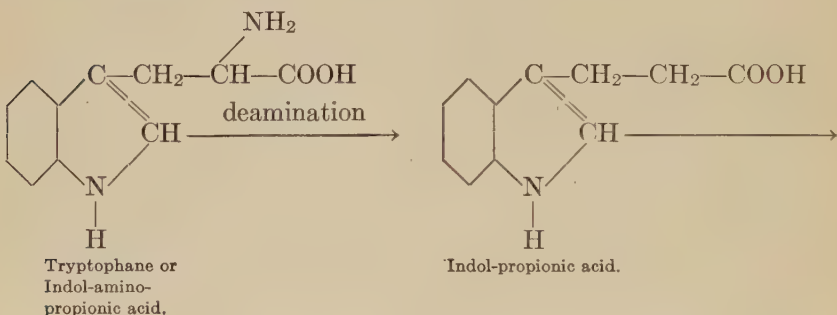
II. Decarboxylation.



III. Oxidation.



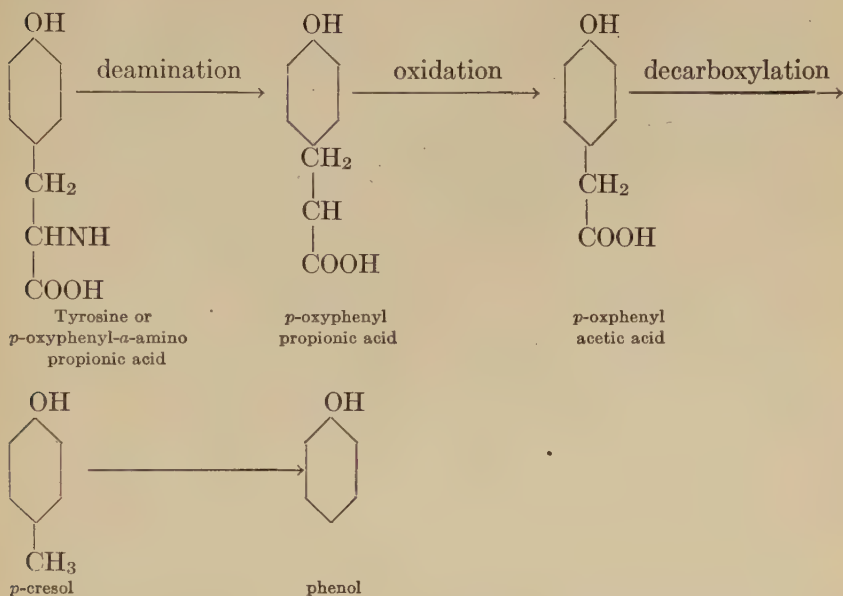
One important characteristic of putrefaction is the malodorous compounds. Their origin may be shown in the decomposition of the amino acid tryptophane.



Indol is one of the commonest products of putrefaction; the bacteriologist examines certain media for the presence of this compound. These tests are given in "Practical Bacteriology" by the author.

The other amino acid usually discussed in putrefaction is tyrosine.

This amino acid, also, gives compounds on decomposition which are malodorous.



Summary of Nitrogen Cycle.—Nitrogen is forced through its cycle by the different forms of life. Of these the bacteria are especially important. Nitrogen makes up 80 per cent of the atmosphere; this nitrogen is available for plant life after it has been fixed by bacteria. This nitrogen appears in plants. The plants may be eaten by animals and the nitrogen either built into animal protein or excreted as ammonia, urea, etc. Or if the plants are not eaten, their bodies fall to the earth where the nitrogen is liberated by putrefaction, denitrification, etc. In all of these changes, which are discussed under the nitrogen cycle, a great amount of energy is involved although the nitrogen compounds are generally looked upon as growth foods rather than energy foods.

THE SULFUR CYCLE

Sulfur is not such an important element perhaps for two reasons. Plants need but a small quantity; and there is enough in ordinary soil for crop requirements. There is no problem in maintaining an adequate amount. The sulfur cycle has some points in common with the nitrogen cycle and also some differences. The plant takes up sulfate from the soil just as it takes up nitrates in the nitrogen cycle. Hydrogen sulfide, like ammonia, may be oxidized to sulfates although it is not known

to take place in two stages as does the oxidation of ammonia. Sulfates may also be reduced like nitrates. The sulfur cycle differs from the nitrogen cycle in the fact that sulfur is not found in the atmosphere.

We will discuss the sulfur metabolism from the standpoint of hydrogen sulfide formation and oxidation.

Production of Hydrogen Sulfide.—This is a common product of putrefaction and its odor along with the odors of other reduced compounds gives putrefaction its bad reputation.

The formation of this substance may be discussed from two angles, its formation from protein and the reduction of sulfates. It is formed in the human alimentary tract and when absorbed may cause illness.

Hydrogen Sulfide from Proteins.—Most of the proteins contain sulfur either in the cystine linkage or some other linkage. When such proteins are decomposed by bacteria under anaerobic conditions, or are putrefied, the sulfur appears in the reduced state as H_2S or $\text{C}_2\text{H}_5\text{SH}$, thio alcohols. There is considerable evidence that hydrogen sulfide results from protein decomposition. It is one of the products contributing to the odor of spoiled eggs; it is also formed in varying amounts in Limburger cheese.

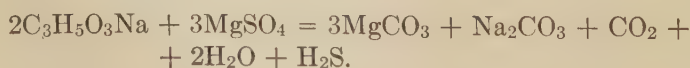


FIG. 103.—*Beggiatoa alba*.
(After Beijerinck.)

a, Filled with sulfur; b, the sulfur partly used up by the lack of the presence of sulfur in the environment; c, the cells almost free from sulfur.

The bacteriologist makes use of lead salts such as the acetate for detecting hydrogen sulfide formation. The lead salts in the presence of hydrogen sulfide give rise to the formation of black lead sulfide.

Hydrogen Sulfide from Sulfates.—The reduction of sulfates to hydrogen sulfide is not a common characteristic among the bacteria. It seems to be a more restricted characteristic than the reduction of nitrates. Only a few organisms have been found which reduce sulfates. Beijerinck and van Delden described two species *Spirillum desulphuricans* and *Microspira aestuarii*. It will be seen after a little thought that sulfate reduction involves the taking away of oxygen from an SO_4 group. This oxygen has to be used for oxidizing some organic substance. Van Delden showed this by using sodium lactate as an example. The following equation was suggested:



Another especially interesting sulfate reducing bacterium has been described by Elion. He called this organism *Vibrio thermodesulfuricans* because it reduced sulfur and was thermophilic. This organism was said to form hydrogen sulfide over night whereas the other two organisms required four or five days.

Oxidation of Hydrogen Sulfide.—Hydrogen sulfide is oxidized by bacteria, according to the following equation, to water and free sulfur.

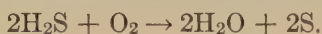
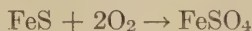
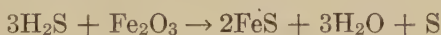


FIG. 104.—*Thiothrix nivea*. (After Winogradsky.)

A, Threads; B, *Thiothrix tenuis*, threads. Note that the threads are firmly attached to the substratum by means of the sucker (at the lower ends).

Fowler stated that this change was hastened by the presence of certain metallic oxides. He gave the following equations using iron to show the steps in the oxidation:



Oxidation of Sulfur.—Sulfur may be oxidized by bacteria to sulfuric acid. Beijerinck and Jacobsen described two species, *Thiobacillus thioparus*, and *Thiobacillus denitrificans* which could oxidize sulfur, sulfids and thiosulfates. In America, Waksman has more recently isolated other forms. He reported *Thiobacillus thiooxydans* which could oxidize elementary sulfur to sulfuric acid; it is especially interesting because it is able to live in an acid medium of pH of 0.6 and less.



Sulfur oxidation is an aerobic process yielding energy for the organism

which carries it on. Such organisms are autotrophic, requiring no organic matter for growth. These organisms are important in the soil for they help to dissolve some of the inorganic matter by means of the acid which they form. This is well illustrated by the composting of sulfur, rock phosphate and soil. The sulfuric acid formed by the sulfur-oxidizing bacteria dissolves the rock phosphate and makes it available for plants.

Another type of sulfur oxidation is possible with *Thiobacillus denitrificans*. This organism is interesting on account of the fact that it is

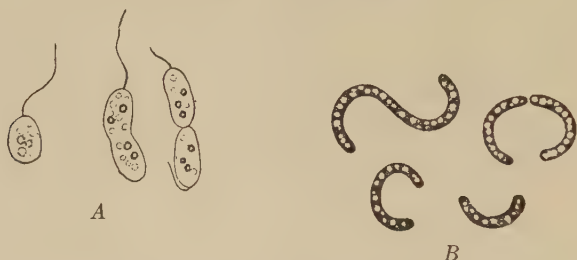
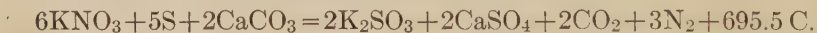


FIG. 105.—A, *Pseudomonas Okenii*. Cells with Sulfur Granules. (After Warming.) B, *Spirillum undula*. Showing the Internal Structure of the Cells. The Vacuoles are White, the Chromatin Granules Black, and the Protoplasm Shaded. (After Fischer.)

able to oxidize free sulfur without an abundant supply of free oxygen. The oxidation is carried out as follows:



This organism takes its oxygen from potassium nitrate and uses it for the oxidation of the sulfur. The sulfuric acid which is formed is neutralized by the calcium carbonate and the potassium.

The oxidation of sulfur has been called sulfication by some soil bacteriologists. This is not a good term since from the Latin words it really means the making of sulfur. However, names are only convenient labels for things and processes and in that respect it is as good a name as any if every one uses it.

Sulfur Bacteria.—This term is perhaps an unfortunate one since it has a restricted meaning. It is generally used for a group of higher bacteria which are especially active in changing sulfur. In the wide sense any bacterium which changes the state of oxidation of sulfur, would be a sulfur bacterium. A discussion of the so-called sulfur bacteria is not necessary here beyond a brief characterization which sets them apart from *Eubacteria*. These sulfur bacteria are divided into several genera which may be discussed briefly.

Beggiatoa.—This genus is characterized by long filaments which are actively motile. The interior of these filaments may be seen to be filled with sulfur granules when the plant is growing in the presence of sulfur. In the absence of a sufficient supply of sulfur, the sulfur granules within the filaments disappear; the thread also segments in a great number of smaller units. Several species are common. *Beggiatoa alba*, *Beggiatoa minima*, *Beggiatoa mirabilis*, *Beggiatoa media*, etc. These sulfur organisms are found in sulfur springs but also in stagnant pools with much organic matter in process of putrefaction.



FIG. 106.—A, *Spirillum sanguineum*. (After Warming.) B, *Clathrocystis roseo-persicina*. (After Zopf.)

Thiothrix.—This genus was established by Winogradsky. Its members consist of long threads and are not only non-motile but are anchored to one spot at one end of the thread. *Thiothrix* is also characterized by a sheath which keeps the threads intact whereas with *Beggiatoa* the filaments break up.

Non-Filamentous Sulfur Bacteria.—These sulfur bacteria, represented by *Chromatium vokenii*, and *Spirillum volutans*, have been studied and confusingly named by a number of different students. They exist as free single cells. These organisms contain a red pigment called bacterio-purpurin. They store sulfur in their cells as a reserve substance, to use it only when the sulfur supply runs out in their environment.

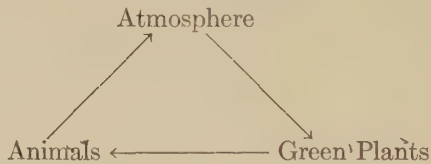
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CHAPTER XVII

THE CARBON CYCLE

Carbon, of course, is a constituent of all kinds of organic matter. To describe its cycle might, therefore, seem unnecessary. The carbon cycle may be started at one of several places. There is, perhaps, a less satisfactory starting place in the carbon cycle than in the nitrogen cycle where atmospheric nitrogen may serve. The carbon cycle is much simpler or, at least, may be expressed in a simpler manner, than the cycles of sulfur, nitrogen, phosphorus, etc. However, it includes very important phenomena. Lotka stated that the organic carbon cycle reduced to its simplest form could be expressed as follows:



This illustration shows the basic living agents which are concerned with moving carbon through its cycle.

Carbon is a widely distributed element on the earth. It is found, of course, in the free state in coal deposits and combined in a great many different chemical compounds. One can scarcely imagine a world without carbon. It does not occur free in the atmosphere as does nitrogen. It is found, however, in small amounts in the atmosphere as carbon dioxide; Greaves stated that it existed in the atmosphere to the extent of 3 parts in 10,000, equivalent to 600 billion tons of carbon. A little thought will reveal that there is a constant interchange of carbon between atmosphere and earth; when coal is burned it is oxidized to carbon dioxide which is poured into the atmosphere. Respiration of animals also adds small amounts of carbon dioxide. Fortunately, nature has provisions for preventing the unlimited increase of carbon dioxide in the atmosphere which would eventually destroy animal life. The compound is utilized by plants for their photosynthetic reactions and there are probably chemical reactions, also, by which carbon dioxide is removed from the air.



Fermentation.—This is probably the most significant process in the carbon cycle. While all the elements which are contained in the molecules fermented are involved, fermentation is usually thought of in connection with the carbon cycle because carbon dioxide is usually formed. There are many definitions of the term fermentation and it has been discussed in other connections in this book. One of the commonest is that fermentation is the decomposition of carbohydrates by living cells.

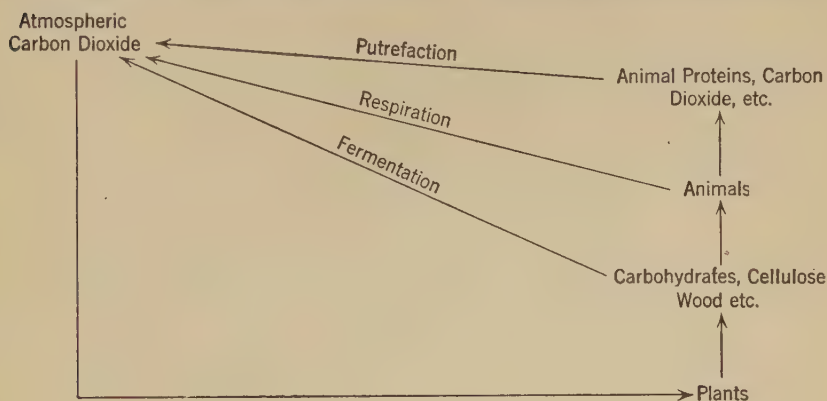


FIG. 107.—Carbon Cycle.

Another term, putrefaction, is used for the decomposition of proteins. Such distinctions are quite permissible since it is frequently impossible to distinguish biological phenomena sharply. Some biologists who work with single-celled microorganisms have defined the term in a different manner. Fischer, for instance, defined it as any intracellular process which furnishes energy to the cell. He gave the following characteristics:

1. Fermentation is an intracellular process.
2. The products of the fermentation are essentially different from those in the substrate and are not mere fragments of these original substances.
3. The products are useless to the cell and may be harmful.
4. Vital energy is produced.

Such a description of the process of fermentation has its merits from the standpoint of microorganisms. It does not distinguish on the basis of the chemical constitution of compounds in the substrate but rather on the basis of the contributions of the process to the cell. According to the above definition it is possible to speak of the fermentation of nitrogenous substances such as urea, proteins, etc. Another definition states that

fermentation is any enzyme reaction. This is a broader definition and has not been widely accepted. The commonest definition, used especially by the biochemists, is that fermentation is the cellular decomposition of carbohydrates.

Lactic Acid Fermentation.—The lactic acid fermentation is the decomposition of carbohydrates with the formation of appreciable amounts of lactic acid. This phenomenon is probably not restricted to bacteria since this acid plays a rôle in the explanation of many biological phenomena. Certain bacterial fermentations, however, cause the formation of larger amounts. Using the lactose molecule, the chemistry of the lactic acid fermentation may be shown as follows:



Such a fermentation is usually brought about by facultative anaerobic bacteria. Considerable energy is left in the molecules of the product since there is not a complete oxidation. The lactic acid fermentation is especially connected with the souring of milk. The bacteria concerned are frequently spoken of as "lactic acid bacteria" because they form larger amounts of lactic acid than the ordinary bacteria.

The chemistry of the lactic acid fermentation is interesting to those concerned with the chemistry of vital phenomena. Lactic acid has an asymmetric carbon atom which permits the existence of two types of lactic acid, a dextro-rotatory type and a laevo-rotatory type. The type formed in bacterial fermentations depends on the species of microorganisms, temperature, etc. The typical lactic acid organism is, among others, *Streptococcus lactis* Lister; it is known under several other names.

Butyric Acid Fermentation.—This acid fermentation is known best by the odor of the acid which is formed. It may be expressed as follows:

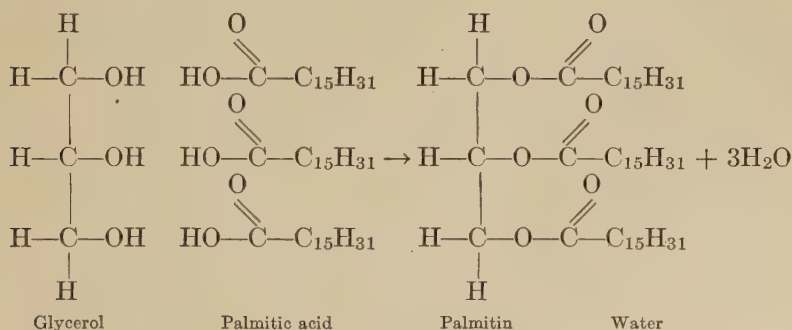


This is the simplest method of expressing it but probably does not show the real changes. Some bacteriologists believe that a number of intermediate reactions take place. These may be added, probably, to give the equation mentioned above. Microorganisms which are able to bring about the butyric acid fermentation are numerous. The most active ones are anaerobic as would be expected since butyric acid is an incompletely oxidized compound. *Clostridium amylobacter* is one species which forms butyric acid in appreciable amounts.

Citric Acid Fermentation.—This acid fermentation seems to be best carried on not by bacteria but by certain molds belonging to the genus *Citromyces*. These molds are much like *Penicillium* species and have been studied by the German mycologist Wehmer. *Citromyces citricus* and *Citromyces Pfefferianus* are the species which are best known as citric acid producers. At present the biological manufacture of citric acid is not a commercially successful venture on account of other cheaper sources of the acid; however, it might not be impossible under certain conditions.

Alcoholic Fermentation.—This is, perhaps, the oldest fermentation in which man has been interested. Fermentations in which the principal product is ethyl alcohol are commonly spoken of as alcoholic fermentations. The production of industrial ethyl alcohol is discussed in a later chapter. The most active alcohol-forming microorganisms are yeasts; a few bacteria have been discovered which form appreciable amounts but less than those formed by the yeasts. The chemistry of alcoholic fermentation has just been explained by Neuberg, a German biochemist. Those who are interested may find the equations in the several texts on alcoholic fermentation.

Decomposition of Fats.—Fats are another group of carbon containing compounds which are of interest. The fats or lipins are esters of glycerol and certain organic acids. When three molecules of palmitic acid unite with one molecule of glycerol, the fat palmitin is secured, as follows:



When fats are decomposed by bacteria, enzymes, or chemical reagents into their constituent substances, the process is known as saponification. The glycerol formed in this process is utilized by bacteria. The fatty acids are also decomposed and used by bacteria. The splitting of fats by bacteria has been offered as one explanation for rancidity in butter and other edible fats.

Decomposition of Carbohydrates.—The carbohydrates, as we have learned, are rich storehouses of energy and building materials for microorganisms. At different places in this book, reference has been made to the action of microorganisms on sugars. In this place the decomposition of a few of the sugars will be considered and attempts will be made to summarize the facts. Sugars are classified in different ways. One of the most usable classifications is based on the chemical complexity of the various carbohydrates. In the following paragraphs the decomposition of these compounds is discussed beginning with the more complex.

Cellulose.—This is one of the most complex carbohydrates; its exact chemical constitution is unknown. The term is probably a collective one applied to a large number of closely related chemical compounds. Cellulose is an important substance in nature. It is found in the cell walls of plants and often appears as the structural material in the stems. Many bacteria and other microorganisms possess cellulase with which they are able to bring about the hydrolysis of cellulose. Some of these microorganisms were studied in the early days of microbiology. Bacteria and chemical agents seem to decompose cellulose in the same manner. It is decomposed through the several simpler sugars. In general, two groups of microorganisms are involved, the aerobic and the anaerobic bacteria.

The aerobic bacteria, since they are able to use atmospheric oxygen, decompose cellulose completely. Less is known about the aerobic cellulose decomposing microorganisms than about those which decompose it anaerobically.

The early work on cellulose decomposition by anaerobic bacteria was concerned mainly with the products of the decomposition. They are known to play an important rôle to-day in the decomposition of cellulose in soil, septic tanks, marshes, etc. Omelianski, who did some pioneer work on cellulose fermentation, discussed the subject from the standpoint of formation of hydrogen and methane. The latter, often spoken of as marsh gas, is formed in marshes where much organic matter is being decomposed as well as in septic tanks where the same reactions are going on. Methane is also formed in the intestines of animals by the cellulose-splitting bacteria which are present. It is well to point out that the ability to decompose cellulose is an important one so far as the carbon cycle is concerned. Those microorganisms which possess cellulase help to prevent elements from being stopped in the cellulose stage. Plant residues are decomposed to compounds which may be fermented by other bacteria. Some have predicted that cellulose fermentation would help solve the problem of a fuel for the internal combustion engine when the gasoline supply has been exhausted.

Starch.—Starch is decomposed by those microorganisms which possess amylase. This enzyme is rather widespread in nature. It brings about the decomposition of starch in a gradual manner. As with cellulose, the simpler sugars are formed. The first step in the fermentation of starch is probably the formation of soluble starch and dextrin. It is believed that a little glucose is formed with each step. After dextrin, maltose is formed and the maltose is fermented to glucose by various common bacteria. Besides these better known decomposition products there are special ones formed by special bacteria. For instance, in another chapter the formation of acetone has been discussed; the principal raw material is corn starch. Alcohol is made by the fermentation of potato starch, although in the latter case the first step in the decomposition may be brought about by hydrolysis with chemical reagents. Like cellulose, starch is a rich source of energy.

Pectin.—Pectins are closely related to cellulose; the term is a collective one used to designate a number of closely related chemical substances. The fermentation of pectin and pectoses is important in the decompositions on which depends the retting of flax. Pectins appear in the flax stem between the cellulose fibers. Flax is subjected to anaerobic fermentation in order to get rid of the pectin binders. Pectin-fermenting bacteria also play a rôle in certain of the soft rots of plants and vegetables.

Other Carbohydrates.—The disaccharides and monosaccharides are easily decomposed by most bacteria. There are a few which seem to be devoid of enzymes with which to decompose certain sugars. We need not mention specific species nor discuss at great length the chemical reactions involved. The more complex sugars are always broken up into simpler sugars; the latter are fragmented to various products many of which are discussed at different places in the book.

Cycles of Other Elements.—More space has been given to the nitrogen, sulfur and carbon cycles than need be given to the phosphorus, arsenic, and other cycles. Phosphorus is present in the soil in several compounds. Many of these compounds are insoluble in water but are rendered soluble by bacterial activity. This may take place by the formation of acids by the oxidation of sulfur, for instance, which, in turn, decomposed the insoluble phosphorus compounds. The path which phosphorus travels in its cycle is not unlike that for the other elements. All forms of living cells help to keep the phosphorus atoms moving along their cycle.

CHAPTER XVIII

PHYSICAL PRODUCTS OF METABOLISM

Various products of metabolism have been discussed at different places in this book. In this chapter attention will be given to some products of metabolism which have a different significance than those discussed in the next chapter. These may be called the physical products of metabolism.

Formation of Pigments.—Some bacteria form striking pigments in their metabolism; this gives another factor for identification. Most of the common bacteria are white; and, when one is found which is pigmented, that characteristic sets it apart from others. Beijerinck, the Dutch microbiologist, classified pigmented bacteria as follows:

I. *Chromophorous Bacteria*: Those which contain a pigment in the cells for some useful purpose.

II. *Chromoparous Bacteria*: Those which excrete the pigment probably as a waste product. The pigment may be excreted either as a colored product or as the colorless leuco-base. The latter may become colored either in the presence of air or other products. Good examples are *Pseudomonas pyocyaneus* and *Pseudomonas cyanogenus*.

III. *Parachrome Bacteria*: These bacteria retain their pigment in their cells. A good example is *Pseudomonas violaceus* and *Serratia marcescens* (*Bacillus prodigiosus*).

Pigments are of little significance beyond their aid in identification. Some of them have been responsible for the striking coloration of certain foods which caused consternation among early peoples. Harrison, for instance, has studied the history of *Serratia marcescens* (*Bacillus prodigiosus*) and reported that the so-called bloody bread was due to the growth of this microorganism. Other foods besides bread also became "bloody." Harrison's paper will be interesting for those who desire more information.

Light Formation (Luminescence).—Many different forms of life produce light or produce compounds which phosphoresce. This is frequently spoken of as cold light. The bacteria producing these compounds have been found to be present on rotting wood, fish and in sea

water. The intensity of radiation varies greatly with the species as well as with the conditions under which the microorganisms are grown. Some of these bacteria produce so much light that the photographic plate is affected. Molisch even proposed the preparation and use of a lantern in which the source of light was an agar film on which phosphorescent bacteria were growing. This lantern was suggested especially for miners but was never put to practical use. Beijerinck proposed the generic name *Photobacterium* for these organisms. Many of them are halophilic, i.e., require the presence of sodium chloride; this explains their presence on fish and the flesh of other marine animals.

Heat Formation.—Many bacterial decompositions are exothermic. If the heat is formed under conditions that do not allow it to be conducted away from the place where it is formed there may be a marked rise in temperature. If the temperature rises high enough spontaneous combustion or ignition may result. It would be wrong to explain all cases of pronounced rise in temperature in certain organic materials on the basis of the metabolic activities of bacteria and related fungi. Heat probably results in the metabolic activities of most cells at times. The respiration of grain and other vegetable cells might result in the formation of sufficient heat to be dangerous. A few fermentations may be mentioned wherein appreciable amounts of heat are formed.

Vinegar Fermentation.—In the quick vinegar or German method for acetification, the temperature of the generator may rise to such an extent that the vinegar bacteria are destroyed. Consequently the vinegar maker has to control this factor carefully. He does this by slowing up the fermentation and reducing the draught in the generator.

The Heating of Hay.—If hay is stored away while it is damp it will heat in the underlying layers to such an extent that it may ignite. The heat in this case may come from thermogenic bacteria or from the enzymic respiration of the plant cells. Burnt hay has been prepared by piling freshly mown grass into solid heaps. These undergo fermentation and cause the temperature to reach 70° C. The heaps are then opened and the hay cured in the usual manner.

Tobacco Fermentation.—During one stage in the preparation of tobacco, it is subjected to a "sweating" process. The bundles are piled up and allowed to heat. The temperature rises rapidly and may reach 55° or 60° C. The piles are then taken down and the leaf dried.

Manure Fermentation.—That heat is formed in fermenting manure is the basis for the use of manure in hot beds and cold frames. The heating of manure is apparent in the winter time when such heaps steam and do not become frozen in the deeper layers. Manure is also used to keep fresh concrete from freezing.

Silage Fermentation.—The formation of heat in silos depends on the same factors that influence its formation in other cases. Too high a temperature destroys the quality of the silage probably on account of the fact that the necessary bacteria are destroyed.

Aromatic Compounds.—Some bacteria form among their various products of metabolism compounds which have an aromatic odor. These have been described as fruity, nutty, etc. Microorganisms which form desirable odors and aromas have been especially studied in the dairy industry where a desirable odor is beneficial. Such is the case in cream ripening. The use of a microorganism for producing a delicate flavor greatly improves the quality of butter. Some attention has also been given to this problem in the vinegar industry; the presence of a fine aroma in addition to the acetic acid would increase the value and desirability of the vinegar.

Besides these physical products of metabolism there are several other chemical compounds which are of marked industrial importance.

Formation of Gas.—Various gaseous compounds may be formed by microorganisms. The commonest is probably carbon dioxide which is one of the characteristic products of fermentation. Bacteriologists use gas formation as one of the differential tests in the characterization of bacteria. An enlightening experiment is quoted by Morrey in his "Fundamentals of Bacteriology." He packed material taken from the bottom of a pond into a cylinder 5 feet long and 6 inches in diameter. Water was added to within 2 inches of the top. The cylinder was tightly closed after a few days and fitted with a pressure gage. After six months the gage showed a pressure of more than 500 lbs. per sq. in. Morrey believed that this experiment explained the formation of natural gas.

CHAPTER XIX

MICROORGANISMS IN THE AIR

There is no typical air flora. As even the most uninformed would predict, the microorganisms in the air would depend on many variable factors. The microbiology of air is hardly worthy of special attention aside from several interesting discussions which may be brought in. Many different methods have been devised for estimating the microorganism content of air. Most of them involve the use of an instrument called an aeroscope. This is a device through which the air to be examined is drawn with the hope that the microorganisms will be caught on the material within the aeroscope. Such materials as water, glycerol, sand, etc., have been used. The data in the literature are somewhat contradictory. By selecting data a person may prove almost any position which he wishes to assume. Consequently, data must be interpreted with judgment.

Microorganisms Present in Air.—This is strictly dependent upon the place where the air is collected. In ordinary country air a diverse flora would be found which would include most of the common saprophytic microorganisms. These would consist, mainly, of the various members of the *Bacillus subtilis* group the spores of which are resistant enough to persist for some time. Coccus forms are also abundant since plates which are exposed to air contamination often show great numbers of colored coccus forms.

Some of the earliest work on the bacteria in air was carried out by Pasteur who was busily engaged in disproving the theory of spontaneous generation. In his argument with Pouchet, Pasteur maintained that the air might contain cells of living organisms which his antagonists claimed developed spontaneously. Pasteur carried out experiments with flasks containing sterile media. He found that air contained bacteria although under certain conditions it might be sterile. Furthermore, he found that the higher the altitude the smaller was the number of bacteria. This work has been carried on by many others. A contemporary of Pasteur, Tyndall, also made experiments. He opened flasks on a glacier but secured no inoculation or infection; however, flasks which were exposed to air in a hayloft were infected.

Important studies on the bacterial content of air have been made in connection with the production of milk with a low bacterial content and the relation thereto of bacteria in stable air. Investigators at the New York Agricultural Experiment Station found between 50 and 200 bacteria per liter of air.

Miquel's data are also usually quoted. One cubic foot of air in Paris was found to contain 150 bacteria; after a rain only 6 were found. A gram of house dust contained 2,100,000 bacteria.

Numerous other data could be reviewed. What conclusions are to be reached? It has been found that expired air is almost free from bacteria although the air taken in may be well populated. The microorganism content varies with the source of the sample and conditions which obtained when the sample was taken.

Relation between Dust and the Presence of Microorganisms in Air.—

On account of the fact that dust particles may often be seen in the air many have connected them with the dissemination of harmful bacteria. The relationship seems to be very indirect, if there is any at all. Here again the kind of dust is the important influencing factor.

In the investigations of the bacterial content of stable air, already referred to, attention was given this phase of the discussion. One paragraph may be quoted.

"In order to get a much higher germ content in the air than occurred in the air of the station stable even under the worst of the normal conditions, a large number of tests were made in the stable loft. Here it was easily possible, by sweeping up debris from the floor, to secure dusty conditions which were as bad as the worst possible conditions obtainable in commercial dairies. When a heavy dust was raised at the beginning of each test, the germ content of the air was usually between 1000 and 2000 per liter, with an average of 2068, a minimum of 239 and a maximum of 5200 per liter. When the dust was maintained continuously throughout the test, the numbers obtained in the completely satisfactory determinations averaged 9575 bacteria per liter of air, with a minimum of 960, a maximum of 28,200, and a usual range from 2500 to 10,000 per liter."

There need be no direct relation between the amount of dust and the number of bacteria. Some dust might be sterile while other dust might be heavily laden with microorganisms.

Winslow and Kligler reported 49,200,000 microorganisms per gram in street dust of New York City and only 3-5 millions per gram of indoor dust. The flora of street dust included 51,000 colon organisms and 42,500 streptococci.

Whipple, who studied the relation between dust and bacteria in air

of cities, found a great variation depending on the place where the samples were collected.

Cleaning Efficiency of Vacuum Sweepers.—The vacuum sweeper is a valuable addition to the equipment in the home as far as the removal of dust and dirt is concerned. Not much work has been done on the ability of these instruments to change the bacterial population in a room. Frost and Armstrong reported such a study in 1911. They worked with both the ordinary portable types as well as with those permanently installed. The latter were said to be more efficient than the portable. The portable types were found to vary greatly in efficiency. In the permanently installed types the exhaust takes the bacteria out of the room and if this exhaust is properly located they become of no significance. A different situation exists with the portable types. The bacteria are not removed from the room but are entrained in a bag of varying efficiency. In most cases Frost and Armstrong stated that it was impossible to prevent the bacteria from getting back into the room since with some of the machines examined the bacteria passed through the cloth bag very rapidly. These investigators used *Serratia marcescens* (*Bacillus prodigiosus*) as the test organism. It is possible that the vacuum sweepers in use today are more efficient.

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CHAPTER XX

WATER BACTERIOLOGY

Water is often the first thing to be blamed in a community or family when an unusual amount of sickness appears. Often this is done without adequate investigation. An abundant supply of safe water is one of the greatest benefits that may be bestowed on a city. The great cities of our country owe their locations to-day, in many cases, to an abundant supply of water; for quantity must be considered first, quality after. Rome, during the Golden Age of her history, is said to have used 400,000,000 gallons of water a day in her baths, fountains and cisterns. Marvelous aqueducts, some of which are being used to-day, brought this water to the city where it was stored in large basins and pools. After the water had been brought to the city, however, it was necessary to carry it to the homes. Rome did not have the great distribution systems possessed by modern cities. The waterworks of the present day are far more complex. We have expensive treatment plants, pumping stations and distribution systems that are marvelous for their utility.

Definitions and Types of Water.—From the standpoint of the relation to disease and illness, water is classified and described in different ways. It will be profitable to define some of these terms in so far as definitions are possible. Pure water, from the standpoint of health, is water which contains no disease-producing bacteria or other harmful matter. Safe water is water which has enjoyed a good reputation for some time and which yields good results on laboratory examination. An impure water has been defined as water which contains either substances or organisms which will disturb the functions of the body and cause illness. Contaminated water is water which contains sewage. It is not necessarily harmful. It is potentially dangerous. Infected water contains disease-producing bacteria. These are a few of the terms which have crept into usage; they are, of course, somewhat arbitrary and impossible to define without overlapping. The consumer desires to know whether the water is safe for drinking and he cares little about the different terms that may be used.

Relation of Water to Disease.—The relation of bad water to disease and illness needs no special emphasis. The relationship has been

admitted since the days when man began to consider causes and effects. However, even in modern times, it has required great epidemics of disease with their attendant death rates and suffering to focus attention on an unsatisfactory water supply. Many of the large cities along the Great Lakes have had to suffer such epidemics before adequate attention was given to water filtration and sterilization. Many of the great scourges which swept over Middle and Southern Europe in the past centuries were caused by dangerous water.

Longevity of Disease Bacteria in Water.—The length of time that disease bacteria live in water is of great importance. If the organisms do not live long the danger is somewhat lessened; on the other hand, if an organism not only lives for a long time but multiplies, the danger is greater. Probably most of the bacteria concerned with the so-called water-borne diseases are not very viable in water and die out in a short time. Too long a discussion would be required to analyze the situation for all diseases which may be caused by water-borne bacteria. Consequently we focus attention on typhoid fever and its etiologic agent, *Eberthella typhi* (*Bacterium typhosum*).

It may be stated that *Eberthella typhi* (*Bacterium typhosum*) probably does not live in natural waters beyond a few weeks. The data from several experiments indicate a much shorter time. This organism also seems to live longer in clean water than in water heavily laden with organic matter. The longevity of this bacterium has been studied in investigations on stream pollution. Most of our data agree that the organism does not multiply in water but decreases so that it would not be expected in water that had been polluted in the remote past. Less information is available for the other bacteria; however, for those bacteria which are allied to *Eberthella typhi*, the data would probably not differ greatly. Certainly such microorganisms as *Corynebacterium diphtheriae*, *Neisseria gonorrhoeae*, etc., would die out very quickly, for they are unable to endure unfavorable conditions.

Evidences of Pollution.—How are we to know that water is polluted and unsafe for drinking? It is easy to formulate definitions and standards, but somewhat more difficult to apply them for judging the quality of a water and equally difficult to interpret the results. The layman is led to doubt the safety of his water supply only when it has some very evident abnormal characteristic such as a bad odor or turbid condition. If it is clear and cool, he may be satisfied, and yet such water may be most dangerous. The sanitarian, however, uses sounder criteria for judging quality. He knows that a water which has all criteria of high quality such as coolness, clarity and tastelessness, may be most dangerous. He appreciates the fact that laboratory examinations may be

necessary to detect the most dangerous type of contamination. It will be profitable now to discuss briefly those laboratory tests which are made in the examination of water and to secure the bases for the interpretations which the sanitarian makes. It would be out of place to go into the detailed laboratory procedures for they are discussed in more advanced texts.

Sanitary Inspection.—This involves the use of common sense in judging the quality of water. Few would expect that the water in a river receiving much sewage would be safe for drinking, or that the water from a shallow dug well in a barnyard could be classed as a safe water. It would be quite unnecessary to go to the expense of having a laboratory examination. In these instances one would expect evidences of pollution. However, there may be cases where evidences of pollution are not so apparent and where a *sanitary inspection* by a qualified engineer or sanitarian might reveal possible sources of pollution which could be corrected before the laboratory examination was attempted. Thus is it seen that sanitary inspection should precede laboratory examination of water to seek information on quality and that it is usually best to leave it to an experienced individual.

Chemical Methods.—These include what are known as the sanitary chemical methods for the examination of water. We will not have space for a detailed discussion of them, but will present only the arguments upon which the chemist bases his methods and interpretations.

Turbidity, Color and Odor.—A good drinking water should, along with other requirements, be odorless, colorless, and tasteless. These determinations are the first that were probably used by early man in judging the quality of a water-supply. He learned in a general way that illness might follow the use of a water with unsatisfactory characteristics and probably began to exclude such waters. Such tests are often spoken of as organo-leptic tests and are among the first used by analysts. The objections to this type of examination are apparent.

Hardness.—A hard water is undesirable and may cause severe intestinal disturbances among people not accustomed to it. There are also a few data which suggest that the continued use of hard water may be harmful.

Chlorides.—Urine contains about 5000 parts per million (mg. per liter) chlorine as chlorides, and consequently a high amount in water is suggestive that sewage has entered. This determination has to be interpreted with care, however, since salt deposits or ocean sprays may cause increased amounts of chlorine.

Reaction.—Most waters are alkaline and this test is usually spoken of as alkalinity determination. An acid water may indicate undesirable conditions

somewhere and should be investigated. Industrial wastes are frequently acid.

Oxygen Consuming Capacity.—As the term indicates, this refers to the amount of oxygen required to oxidize the organic matter in the water. Generally speaking, this will indicate the amount of sewage which has reached the water.

Ammonia, Free.—This is ammonia which is in free or saline condition. A high amount of free ammonia may indicate much sewage, although there are often other circumstances which explain it.

Ammonia, Combined.—This nitrogen is combined in undecomposed compounds. This determination generally indicates the amount of decomposing matter.

Nitrites.—A high amount of nitrites may indicate pollution or that decomposition is active. It indicates more remote pollution in respect both to time and distance than the ammonia determinations.

Nitrates.—High nitrogen as nitrates indicates that much organic matter has undergone decomposition and that considerable time has elapsed for the oxidation of reduced nitrogen to oxidized nitrogen.

Bacteriological Methods.—The bacteriological methods are somewhat more direct since they deal with just those agents which cause communicable disease. Standards of quality have to be established by the methods that are used, before these methods may be used for separating good water from bad water. If the chemical methods are used, they must be founded not only on standard procedures for carrying out the tests, but also on standards for interpreting the results. Consequently, in America, these standard procedures for water are embodied in a book entitled *Standard Methods for the Examination of Water and Sewage* published by the American Public Health Association. This book is revised about every five years and therefore represents the accepted practice. Regardless of the methods which are used for judging the quality of a food or water, some factor must be selected which may serve as the indicator of pollution. While a number have been proposed and studied, in the following paragraphs only the most important one will be discussed. It will be seen that some of the bacteriologists' methods are somewhat circumstantial and indirect. Most of them, however, have sufficient years of use behind them to make them fairly reliable.

Escherichia coli (Bacterium coli) as an Indicator of Pollution.—This organism has become significant in sanitary work where it serves as an indicator of fecal pollution of foods and water. A better understanding of the present position of this organism is secured by reviewing briefly its past history and some of the experiments which have brought the organism to its present significance. The organism is found in our

literature under different names,¹ depending on the classification which the author was using. It is also probable that it has been confused with other closely related forms which, as will be shown later, are now separable from one another by new methods.

The species was discovered by Escherich in 1884, in the feces of a cholera patient, and was at first thought to be the cause of the disease. Further work indicated, however, that it was also present in the intestinal tracts of normal individuals. On account of its presence in fecal matter, its presence in sewage was admitted. Consequently, when it was found in water or on foods, it was agreed that such food or water had been in contact with sewage or fecal matter. *Escherichia coli* (*Bacterium coli*), in this manner, secured its reputation as an indicator of pollution. Thus it was used for a number of years, until reports began to be made that the organism, or one very much like it, was being isolated from water and foods which were known to have a clean history and not to have received evident contamination with sewage or fecal matter. Filter operators and others concerned with the treatment of water began to ask bacteriologists how they could use an organism as an indicator of pollution, which at times could be found in water known to be clean. This caused bacteriologists to seek data for either defending their position or correcting their methods of analysis and standards.

They sought to answer the question in a very logical and reasonable manner. They decided to isolate from various known sources of pollution such as sewage, excreta from different animals, etc., a series of cultures for intensive study to determine whether these strains possessed characteristics which separated them from apparently similar organisms from other sources. Another series of cultures was isolated from unpolluted sources such as grain heads, mountain streams, etc. Then, an intensive study was made of both series and the results compared. This indicated that bacteriologists had been working with and confusing two very closely related organisms. It may not be profitable to outline briefly the several steps used in the separation of these two organisms. These have resulted in the establishment of two types which are often spoken of as the *grain type* representative of unpolluted sources, and the *fecal type* representative of polluted sources. The former, the grain

¹ In bacteriological literature the organism appears under the names *B. coli*, *Bacterium coli*, *Bacillus colon*, *Escherichia coli*, etc., depending on the classification which was used. In this chapter both the old and new names are given since it is believed that a student will be better able to study into the subject than if only the old names were used. Certainly knowledge with regard to the true situation is more to be desired than ignorance or unfamiliarity.

type, became known as *Aerobacter aerogenes* (*Bacterium aerogenes*) and the latter, the fecal type, as *Escherichia coli* (*Bacterium coli*).

The first step in the differentiation of these two types was to determine the ratio of the amount of hydrogen formed to the amount of carbon dioxide. The fecal type, *Escherichia coli* (*Bacterium coli*), formed equal amounts of carbon dioxide and hydrogen. The non-fecal or grain type, *Aerobacter aerogenes* (*Bacterium aerogenes*), formed twice as much carbon dioxide as hydrogen. While this method was an accurate and usable one for research work, it was not suitable for routine work where results are desired as quickly as possible without long laborious technic.

The second step involved the discovery that the fecal type, *Escherichia coli* (*Bacterium coli*), produced a lower hydrogen-ion concentration than did the non-fecal or grain type, *Aerobacter aerogenes* (*Bacterium aerogenes*). This means of differentiation involved the use of an expensive apparatus for determining the hydrogen-ion concentration electrometrically. Such apparatus is not only expensive, but a single observation requires considerable time and specially trained workers. This excluded the method from routine practice. Finally, it was suggested that an indicator similar to those used in the chemical laboratory could be used for following the hydrogen-ion concentration of the cultures. An indicator had to be selected which changed color at that point on the scale where these two organisms functioned. Methyl red was selected, for it was deep red at a hydrogen-ion concentration produced by the fecal type (*Escherichia coli*) and a yellow at a hydrogen-ion concentration produced by the grain or non-fecal type (*Aerobacter aerogenes*). Having established this with pure cultures of known origin, the indicator could then be used for classifying unknown strains.

The third step in the differentiation of these two types of organisms is based on their formation or lack of formation of acetyl-methyl-carbinol. The demonstration of the presence of acetyl-methyl-carbinol has become known as the Voges-Proskauer reaction. It is carried out by putting a little sodium hydroxide into a lactose broth fermentation tube in which the organism has grown. The formation of a red color is a positive Voges-Proskauer reaction and no change in color indicates a negative reaction. By studying bacteria from two sources, unpolluted and polluted, it was shown that the fecal type, *Escherichia coli* (*Bacterium coli*), was Voges Proskauer (-) minus and that the non-fecal or grain type, *Aerobacter aerogenes* (*Bacterium aerogenes*), was Voges-Proskauer (+) plus.

These characteristics may then be summarized in the following manner:

Fecal Type <i>Escherichia coli</i> (<i>Bacterium coli</i>)	Non-fecal or Grain Type <i>Aerobacter aerogenes</i> (<i>Bacterium aerogenes</i>)
1. Found in feces, etc. 2. Forms equal amounts of CO₂ and H in lactose broth 3. Causes a lower hydrogen-ion concentration 4. Methyl red + Voges-Proskauer —	1. Found on grains, etc. 2. Forms twice as much CO₂ as H in lactose media. 3. Causes a higher hydrogen-ion concentration 4. Methyl red — Voges-Proskauer +

This tells us how *Escherichia coli* (*Bacterium coli*) became an indicator of pollution and what things are kept in mind when water is examined for it. *Escherichia coli* does not ordinarily produce disease when taken into the intestinal tract. Its presence in water indicates the presence of sewage or other undesirable matter. When *Escherichia coli* (*Bacterium coli*) is present, *Eberthella typhi* (*Bacterium typhosum*) may be present, for both of these organisms may be found together in sewage when the sewer has received excreta from a typhoid patient. Consequently, when *Escherichia coli* is present, *Eberthella typhi* may use the same channel to reach the water supply.

It may also be pointed out here that it is usually too late to analyze water to determine the cause of typhoid fever after it has appeared in a community. It takes about fourteen days for the symptoms to appear after a person has been infected. Consequently, during that interval, the organisms may have disappeared and the water supply might show no evidences of pollution.

Method of Purification of Public Supplies.—Few large bodies of water are clean enough to furnish drinking water which can be used without treatment. Some cities have purchased small lakes, moved off the habitations from the watersheds and by a system of sanitary inspection and patrol are maintaining a reasonably satisfactory situation without the elaborate system of treatment outlined below. The efficiency of this, however, depends on so many variable factors that most cities have insured themselves against polluted water by resorting to, at least, disinfection. This is the safest procedure since there are many opportunities for contamination even under the best systems of control.

Plain Sedimentation.—In this step, the water is conducted into large basins or reservoirs where nature is allowed to do what she will toward getting out as much foreign matter as possible. This step also has a leveling effect on the quality by tending to make the water to be handled later more even in constitution. The water is conducted from the river, lake, or other source, into large sedimentation basins where it is

allowed to stand quietly or by means of baffles is made to travel slowly over a long route through one or two basins. Much of the heavier suspended matter is removed in this way.

Sedimentation with Coagulation.—From the plain sedimentation basins, the water is conducted to “coagulation basins” where a coagulant is added. This coagulant is a chemical which, in water, is hydrolyzed to a flaky (flocculent) compound. This latter compound floats in the water in large flakes. When the velocity of the flow is lowered, these flakes settle out and carry with them foreign matter which was not removed in the plain sedimentation basin. Iron and aluminum sulfates are the commonest materials used as coagulants. We may illustrate the chemical changes with aluminum, for instance, as follows:



The aluminum hydroxide comes down as a very flocculent precipitate. When alum was first added to water, questions about its possible poisonous effect arose in the public mind. It was shown that the chances of poisoning were very remote, for most of the alum is removed on the filters.

Filtration.—Since the two steps just discussed will not remove all of the foreign matter from water it is next passed to sand filters, of which there are two kinds or types. These are separated according to their nominal capacity or the rate at which water passes through. The filters are usually large concrete basins in the bottom of which there are about 9 inches of gravel and 30 inches of sand. Beneath this material are the pipes for carrying away the water that has percolated through the sand.

Slow Sand Filters: These filters are constructed as described in the above paragraph. They are large, perhaps an acre in size. After they have been used for a while the water comes through so slowly that they are inefficient and must be cleaned. The top 4 or 6 inches of sand with accumulated dirt is removed, washed and replaced. These filters deliver about 3,000,000 gallons of water per acre per day.

Rapid Sand or Mechanical Filters: Rapid sand filters handle about 125,000,000 gallons of water per acre per day. They differ from the slow sand filter by having a mechanical system for cleaning the sand. Underneath the gravel are two other systems of pipes besides the underdrains mentioned above for slow sand filters. One delivers clean water to the bottom of the filter and the other compressed air. When the filter has become inefficient on account of accumulation of too much foreign matter on the surface the sand is washed by pumping clean water back-

ward through the filter. In order to break up the sand, compressed air is also pumped through with the clean water. The clean water washes the sand and carries the dirt with it to the sewer.

The action of filters may be explained in different ways. First, they catch on their surfaces the foreign matter in the water. This gives a thick coating on the surface which is called the "jelly layer." In this are great numbers of protozoa which feed upon the bacteria that are strained out of the water. This biological process probably best explains

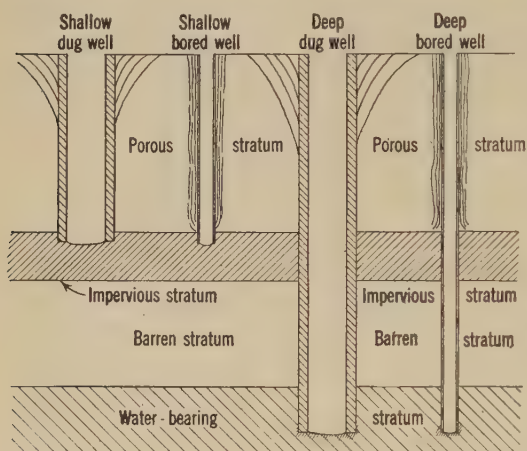


FIG. 108. — Showing the Construction of Dug, Driven, and Bored Wells. This Indicates the Greater Safety of Drilled Wells Over Dug Wells.
(After Bartow.)

the action of filtration in water purification. While in most cases undesirable bacteria might be removed by filtration, there might be an occasional time when they would pass through. This has caused sanitary engineers to follow filtration by a sort of finishing treatment, disinfection.

Disinfection.—After water has passed through the steps just outlined, it is disinfected to allow a greater margin of safety and to make the water as safe as possible. For this

purpose the chlorine compounds have been most widely used. Calcium hypochlorite, which was formerly used, has been replaced by liquid chlorine. The actions of these compounds in general disinfection has been discussed in a former chapter and need not be gone over again here. For water disinfection the chlorine compounds approach closely the ideal disinfectant. They have been responsible for a marked reduction in the incidence and mortality of typhoid fever.

Household Filters.—These have been offered from time to time for the treatment of water for households in cities where municipally treated water is not available. Generally speaking, they are not dependable and may often cause those who use them to live under a false sense of protection. They should not be allowed to replace adequate disinfection. Experiments have been made which showed that the bacteria causing typhoid fever, cholera, and certain other bacteria could pass through these filters. One extensive investigation involving over

1000 household filters was made in Chicago in 1914 and revealed that one should not place too much confidence in this type of filter.

Home Water Supplies (Rural Supplies).—Where adequately treated city water is not available, a great amount of trouble may often be necessary to secure enough pure water. Rural communities and country homes generally rely on one of several possible sources, some of which we will now consider.

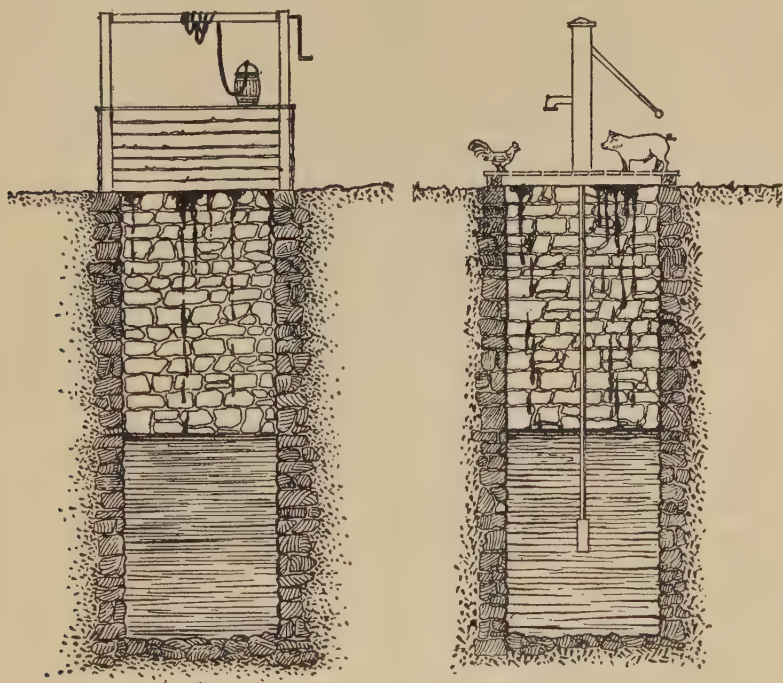


FIG. 109.—Showing Dug Wells Not Adequately Protected from Pollution. (After Hansen.)

Dug, Drilled and Driven Wells.—The dug well is a common source of water for rural homes. It may vary in depth and be lined in different ways. It is often open to serious criticism on account of its location. It should not be located where drainage water may run toward it nor should it be located near privy vaults or barnyards. Underground channels may exist through which pollution may reach the well; even though the water seems to be of good quality and appearance, this pollution may be dangerous. A drilled well is made by forcing an iron pipe or casing into the ground. When a water-bearing area is reached the

drilling is stopped. Such water is usually of good quality although leaky casings have been known to allow pollution. Driven or drilled wells are to be preferred to dug wells. The latter, on account of porous sides, are

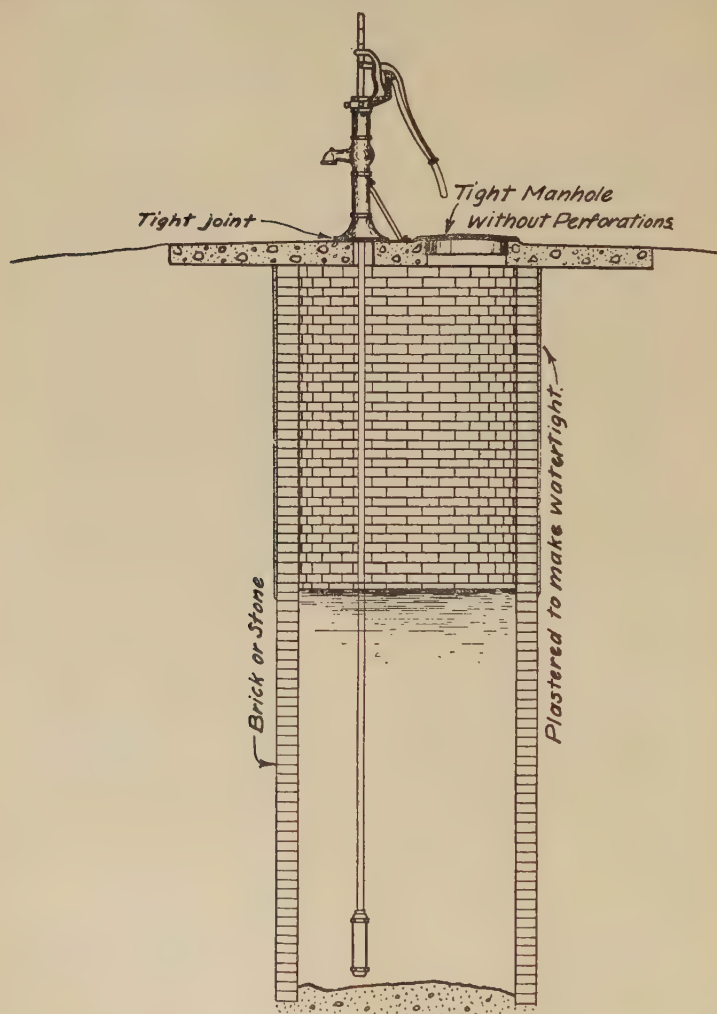


FIG. 110.—Dug Well Adequately Protected against Surface Pollution. (After Hansen.)

liable to receive drainage water. This is impossible with a properly cased driven or bored well. The water from these wells has had to pass through a layer of earth which acts as a filter. Drilled wells often

pass through a stratum that is impervious to water. This means that the water was collected on some distant watershed and has had to flow some distance under the ground.

Wells must be properly constructed and be adequately protected from contamination. Especially is this true of the dug well which may allow surface water to run back in. Cesspools near such wells are dangerous for they usually contain much water that must percolate away through the soil. The distance allowable between a cesspool and well depends on several factors such as the height of ground water, the type of soil, etc. When the ground water is high the distance should be greater between a source of pollution and the well. Consequently, it is far better to put wells deep enough so that there is an impervious stratum above the place from which the water is pumped.

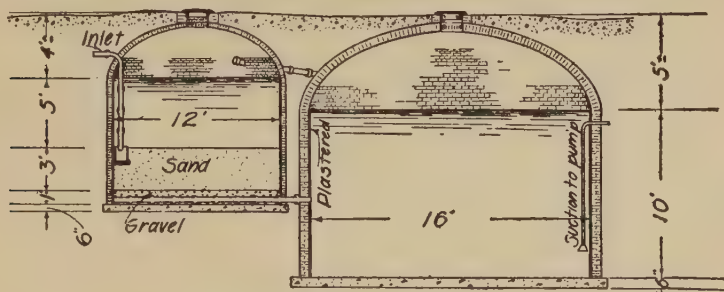


FIG. 111.—Suggested Design for a Sand Filter and Cistern Suitable for a Home.
(Illinois State Water Survey.)

Springs.—Water from springs is an underground water and, if properly protected from surface pollution, is safe. Water from springs is regarded by some as pure and by others as open to contamination. The geological strata which cause ground water to flow to the surface may be such that contamination of the water may occur. To a certain extent, spring waters are filtered water, although the filtration may not be controlled. The number of bacteria would vary greatly and be dependent on the contamination that might occur after the water had come to the surface.

Cisterns.—The cistern in certain communities is the main source of water. Rain water from roofs is usually the original source. Cisterns furnish a satisfactory water as long as the cistern is properly constructed to prevent the ingress of surface water and dirt about the top. A properly constructed cistern is shown in Fig. 111.

Disinfection of Small Quantities of Water.—It is often desirable to render a small quantity of water safe for drinking and culinary purposes.

Such occasions arise on camping trips, vacations, etc. For this purpose, we may quote some instructions from the Illinois Department of Public Health.

Water from a well, cistern, or spring which is suspected of being contaminated should be sterilized before it is used for drinking or culinary purposes. The easiest and surest method is to boil the water. It is not necessary to boil for any great length of time, but be sure that it comes to a distinct boil. The flat taste which results may be partially removed by pouring the water from one vessel into another several times, or by adding a pinch of salt.

Because it is not convenient to boil a large amount of water, it is sometimes more desirable to sterilize the entire well or other supply by the use of calcium hypochlorite, commonly known as chloride of lime, chlorinated lime, or bleaching powder. This chemical can be purchased at any local drug store in small sealed tins. Obtain a fresh supply if possible, because the chemical deteriorates somewhat upon standing even though sealed. When the can is opened a decidedly pungent odor should be evident.

Add one ounce of chloride of lime for each 1000 gallons of water to be treated. If scales are not available the material can be measured with a spoon. A moderately heaping tablespoonful of chloride of lime (that is with the powder about 1 inch deep in the center of a spoon) weighs approximately 1 ounce. The amount of water present in a circular well or cistern can be determined from the following table:

Diameter of well in feet.....	1	2	3	4	5	6	7	8
Gals. for each foot of water in well...	6	24	53	94	147	212	288	376

Rub up the dry powder with a small amount of water to make a thin paste, taking care to break up all lumps, and stir this paste into a bucketful of water. This had best be done out-of-doors to avoid the chlorine fumes which are evolved. Pour the contents of the bucket into the well and if possible agitate the water with a clean board to insure thorough mixing. Allow the water to stand for a period of several hours before using.

The above treatment corresponds to a dosage of 2 parts per million of available chlorine and should impart a slight taste to the water, but this taste is entirely harmless and only serves to indicate that sufficient chlorine has been added to sterilize the water adequately. In fact unless a pungent odor or taste is evident, repeat the treatment using one-half the dosage indicated. It must be borne in mind that such a procedure will sterilize only the water which is actually present in the well at the time of treatment. If the well is subject to seepage from the surface or from a source of pollution such as a privy vault or sewer, the treatment should be repeated as often as a quantity of water equal to the capacity of the well has been pumped out. Any process of sterilization is at best only a temporary measure, and immediate steps should be taken to reconstruct the well so that it will be protected against further contamination.

For sterilizing small quantities of water rub up a moderately heaping teaspoonful of chloride of lime with a small amount of water in the manner indicated above and add sufficient water to make a pint. Of this solution use one tablespoonful for each ten gallons of water to be treated, or 36 drops to the gallon.

The U. S. Army Medical Corps has found that two drops of ordinary tincture of iodine (7 per cent) added to a quart of water will destroy all disease germs and render the water safe for drinking purposes in one-half hour.

How to Judge Quality of Private Water Supplies.—This is not always an easy task. While the physical appearance, as we have learned, is not always a safe criterion, it does yield some information. Laboratory examination is the safest method. This involves, as we have learned above, an examination for fecal *Escherichia coli* (*Bacterium coli*). Great care must be used to collect the specimen for analysis. The sample may be contaminated during collection when the original water was in satisfactory condition. A single analysis is not conclusive. Several examinations enable a person to arrive at a better opinion of the quality of water. A sanitary inspection is often necessary to find out whether there are open channels which might allow pollution to reach the water supply.

How to Have Water Analyzed.—Most states provide for the sanitary examination of water, either free of charge, or at a very moderate cost. This may be determined, in most cases, by writing to the State Board of Health. There are several points in this connection which are often overlooked by those who seek such help from laboratories. It is usually best not to send a sample to the laboratory in a container which has not come from the laboratory. It is best to write to the laboratory first and state the facts which prompt the request for laboratory examination. Practically all reliable laboratories which examine water rightly refuse at the start to examine water bacteriologically which has been shipped in any sort of a container, even though the sender claims to have thoroughly cleaned and boiled the bottle. He may not have boiled it long enough or he may have contaminated it after boiling. His conception of sterilization is quite different from that of a laboratory worker. If such samples are examined, the laboratory does not know whether satisfactory results are due to the presence of some chemical disinfectant or whether bad results are due to a dirty, unsterilized bottle or the water itself.

Another point is not to ask the laboratory to examine water for typhoid bacilli. It cannot be done readily; Standard Methods for the Examination of Water and Sewage does not provide for this in routine water examination. There are no satisfactory routine procedures, and

even if it could be done the absence of this organism at the time of examination would not prove that the typhoid organism might not appear in the water later. It is better to look for evidences of pollution which show open channels between sewers, barnyards, etc.; this gives more information about possible future pollution.

The last point to be mentioned concerns the information which should accompany the sample. Some persons decline to fill out completely the certificate that is sent with each sample bottle. They feel that if a laboratory worker knows nothing about the source from which the specimen came, he will start his work with no preconceived ideas and therefore his conclusions will be more trustworthy. This is the wrong attitude. The laboratory worker rarely knows the sources of the specimens upon which he works unless he takes the pains to look them up. The specimens go through his hands under numbers. For him to give a trustworthy opinion, he must have all of the information called for on the certificate sent out with each sample bottle.

Bottled and Mineral Waters.—These have enjoyed a wide sale in America because of the prevailing opinion in certain localities that they are better than the municipal supply. The Bureau of Chemistry in Washington several years ago made a careful study of the bacteriological condition of these waters and found much to be desired. They found that some springs were being used for the production of mineral waters which should not be used. Before a spring water is put on the market it is an easy matter to determine whether it is pure and safe for consumption. This may be done by sanitary inspection and laboratory examination. Private homes depending on mineral waters for drinking water should request the vendor to supply a certificate showing that the water has been examined by the local city or state health department.

Bacteriology of Ice.—Two kinds of ice are used in America, natural and artificial. Natural ice is harvested from natural bodies of water in the late winter and stored for sale during the summer. Artificial ice is made the year around and is sold soon after manufacture. The hygienic properties of the latter are obvious and if this ice is properly handled it is safe for consumption. The natural ice, however, needs a little discussion.

When ice crystals form in water they tend to come down in the pure condition. Any impurities that are in the water are excluded. In the chemical laboratory, this method is used for purifying chemicals. Ice, then, even from relatively polluted bodies of water, tends to be found in the pure condition. This indicates that nature does her part toward making ice safe for consumption. Natural ice is usually stored for months during which time the bacterial content would be markedly low-

ered. Jordan stated that after three or four months storage the danger from ice from even highly polluted water would be very slight and after six months storage would be practically negligible. These statements do not condone the harvesting of ice from polluted bodies of water even though such ice has been found to be free from objectionable bacteria. As an added element of safety, ice should be analyzed and certified by competent health authorities.

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CHAPTER XXI

SEWAGE BACTERIOLOGY

~~Sewage has been defined as the used water supply of a community or home.~~ In the preceding chapter the problems of securing a sufficient amount of good water were stressed. In this chapter some of the methods for disposing of this used water are discussed. The difficulties of accomplishing this are apparent to those who have studied the question. The water in sewage serves as a carrier for the wastes of home and community life. The subject of sewage disposal and stream pollution will be of increasing importance in America as the density of population increases in certain urban districts. Intelligent citizens of the future will be informed on these subjects.

Bacteria in Sewage.—The bacteria in sewage are those which were ~~in the water plus those which have been added when the water was used.~~ The flora is, therefore, quite heterogeneous. Ordinary fresh sewage may contain about 3,500,000 bacteria per cubic centimeter although there is great variation as would be expected. Pathogenic bacteria cannot live long in sewage because they are unable to endure the conditions which exist and the antagonistic effect of so many other types. The bacteria in sewage may be grouped in different ways.

Intestinal Bacteria.—These are contributed to the sewage by the use of water in the home for flushing purposes. As has been pointed out before they are of marked sanitary significance and one type, *Escherichia coli* (*Bacterium coli*), is used as an indicator that sewage has reached a water supply or food. The intestinal bacteria are of greater interest than the other groups.

Soil Bacteria.—The soil bacteria are of little significance from the sanitary standpoint. They may be added to sewage by drainage water and from similar sources. They may include, however, some of the types which are desired in the biolysis of sewage.

General Principles in Sewage Treatment.—The problems of sewage disposal center about the disposal of organic matter and the destruction of any pathogenic bacteria which may be present. The organic matter is usually decomposed by bacteria by hydrolysis. This results in the formation of products which are in the reduced conditions such as

hydrogen sulfide, ammonia, mercaptans, etc. Compounds in this reduced condition usually have bad odors and they may become a nuisance. It is necessary to follow hydrolysis by oxidation so that the reduced compounds are changed to a stable state. In this state they are usually odorless. The oxidation of hydrogen sulfide to sulfate, for instance, causes a disappearance of the foul odors. Consequently, sewage treatment comprises first hydrolysis and then oxidation. These changes are expressed by sanitary engineers under the term "biolysis" of sewage. This term means the breaking down of complex material by means of living agents, and it is a very useful one to use. The decomposition may be due to living protoplasm or enzymes.

Rideal has given a tabular outline of the stages which are involved in biolysis of sewage. This is sufficiently important to reproduce. (Table VII.)

TABLE VII

SHOWING THE STAGES INVOLVED IN THE BIOLYSIS OF SEWAGE

(From Kinnicutt, Winslow and Pratt's "Sewage Disposal")

Stages Involved	Substances Dealt With	Characteristic Products
<i>Initial</i> Transient aerobic changes by the oxygen of the water supply rapidly passing to	Urea, ammonia, and easily decomposable matters	
<i>First Stage</i> Anaerobic liquefaction and preparation by hydrolysis	Albuminous matters Cellulose and fiber Fats	Soluble nitrogenous matter Phenol derivatives Gases Ammonia
<i>Second Stage</i> Semianaerobic breakdown of the intermediate dissolved bodies	Amino compounds Fatty acids Dissolved residues Phenolic bodies	Ammonia Nitrites Gases
<i>Third Stage</i> Complete aeration; oxida- tion and nitrification	Ammonia and carbona- ceous residues	Carbon dioxide, water, and nitrates

This table was discussed as follows by Kinnicutt, Winslow and Pratt in their "Sewage Disposal":

1. *Proteins*.—In the first of Rideal's stages, the proteins are broken down into the albumoses and peptones with the separation of sulfur as hydrogen sulfide, or as mercaptans, sulfur alcohols having very disagreeable odors. The albumoses and peptones have a less complex molecular structure than the proteins; they are soluble in water and are not coagulated by heat. These compounds also during the first stage break down into the so-called amino acids, chiefly acids of the fatty hydrocarbon series containing carbon, hydrogen, oxygen, and nitrogen in which one hydrogen atom is replaced by the amino group (NH_2).

The amino acids thus formed are decomposed during the first and second of Rideal's stages, giving ammonia, phenols, fatty and aromatic acids. All of the nitrogen, however, is not converted into ammonia, for part remains united to hydrogen and carbon, forming amines like tri-methyl amine (CH_3)₃N, part is liberated as nitrogen, while a certain portion is undoubtedly directly converted into nitrous acid.

The last change in the process of decomposition is a partial or complete oxidation of the organic substances formed by the decomposition of the amino acids, resulting in the production of water, carbon dioxide, nitrous and nitric acids. Gaseous nitrogen is sometimes liberated in large quantities by the action of amines on nitrous acid.

The principal products of the bacterial action on the proteins are amino acids of the fatty hydrocarbon series; relatively small amounts of the aromatic compounds, such as phenyl alanine, tyrosine, tryptophane, phenol, skatol, and indol, are formed.

2. *Carbohydrates*.—Sugar and starches are very easily hydrolyzed and broken down by the action of bacteria, and though alcohol may be one of the products of the decomposition, the principal substances formed seem to be butyric acid, lactic acid, water, carbon dioxide, and hydrogen. Cellulose and woody fiber are also broken down and liquefied, but comparatively slowly and the action is often so sluggish that they are but little changed in the process of sewage treatment. Hydrolysis plays an important part, at least during the first stages of the decomposition of cellulose, and the products formed are undoubtedly similar to those produced from the sugars and starches.

3. *Fats*.—The decomposition of fats, brought about by the action of bacteria and molds, is again largely a process of hydrolysis, the fatty acids, stearic, palmitic, oleic, butyric, and glycerol, being the chief products. Further hydrolysis converts the fatty acids into carbon dioxide, hydrogen, and methane. The breaking down of the fats takes place very much more slowly than that of proteins, and it appears probable that they must first emulsify before being acted upon by bacteria. The emulsification is at least partly brought about by the ammonia set free in the decomposition of the amino acids. The slow decomposition of fats and greases makes a sewage containing large amounts of these substances very much more difficult to purify than a sewage in which the organic matter is mainly in the form of proteins and carbohydrates.

Preliminary Methods.—These include the methods which are hydrolytic in nature. We need not discuss the other preliminary steps such as screening and the like for they are mainly mechanical. Hydrolyses are brought about mostly by two methods or modifications of these methods.

Septic Tank.—The septic tank is a concrete basin through which the sewage passes slowly; during this time the solid matter settles out and

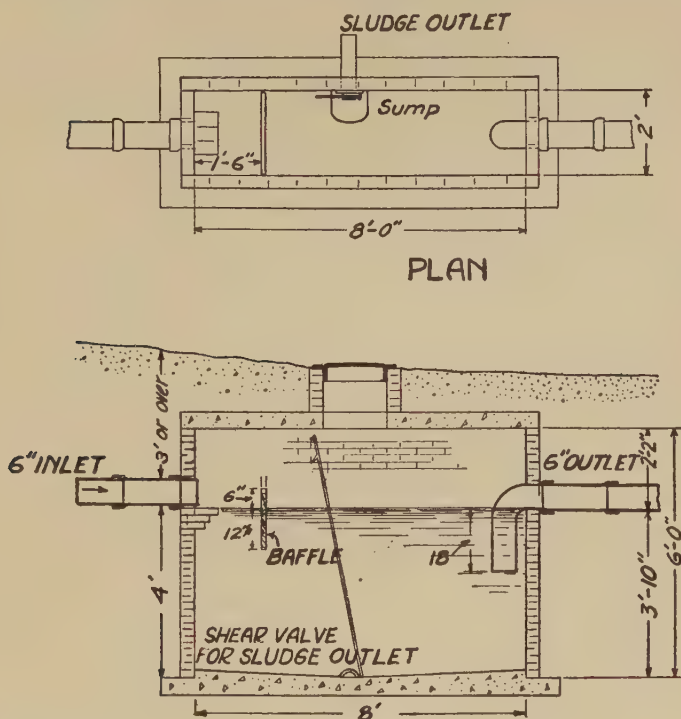


FIG. 112.—Suggested Design for the Simplest Form of Sewage Tank for a Farm Residence or Other Home Not Connected with a Sanitary Sewer. (*Illinois State Water Survey.*)

undergoes anaerobic decomposition at the bottom of the tank. This anaerobic decomposition is one of hydrolysis mainly, and the products must yet be made stable by oxidation. The products of these hydrolyses are the various decomposition products of proteins, carbohydrates, fats, etc. The septic tank is commonly used for disposing of the sewage from rural homes and small communities. These tanks are frequently neglected since the layman believes that they are automatic and operate

in some mysterious manner. The chemical changes which take place in the septic tank are contained in Table VII by Rideal (see p. 311).

The Cesspool.—This is usually a moderately deep hole, the sides of which are laid up with brick or flat stones without mortar. The organic matter is delivered to it and probably undergoes anaerobic decomposition while the water seeps away into the ground. The cesspool would, of course, be satisfactory for single residences or where a small amount of sewage must be cared for. Cesspools should not be used or should be located with discretion where the water for household purposes comes from shallow wells.

Finishing Processes.—Under this heading may be mentioned those processes to which the product from anaerobic processes are subjected. They are, of course, mainly aerobic since they are oxidative in nature. The reduced compounds such as H_2S , NH_2 , etc., which result from the hydrolytic processes must be oxidized to a stable condition. The following methods are available for this purpose.

Dilution.—This is the oldest method for the disposal of sewage. The sewage was usually dumped into a stream below the city and the forces in nature were trusted to take care of it. While this method may have been adequate before the population over the country became as dense as it is to-day, the method long ago reached the limit of usefulness. Its continuation has caused some very bad situations; on account of the seriousness of the question in many places in America to-day, we may be justified in giving it a little more attention.

The disposal of sewage by dilution in a river or other body of water resolves itself into a consideration of three factors: 1, organic matter—the sewage itself; 2, bacteria; and 3, oxygen. The oxygen is necessary for the oxidation of the organic matter. The organic matter and bacteria are always present and may therefore be dropped from our discussion. The question then comes down to a consideration of what may be called the “oxygen balance.” By this is meant the amount of oxygen required for oxidation of organic matter in relation to the amount which is available. Relatively clean river water contains dissolved oxygen and the question arises, how much sewage can a stream carry and oxidize to such a condition that it will not become a nuisance? While the question can be answered for a given stream only by laboratory analysis, it has been suggested that for every gallon of sewage there should be at least 50 gallons of relatively clean river water. The determining factors, however, are the concentration of the sewage, the quality of water in the stream, etc. Such statements are of general significance only.

Stream Pollution.—Discussion of this subject may seem somewhat out of place in a book of this nature. Since it is closely related to sewage

disposal and since it is a subject in which all citizens, especially those who have studied bacteriology, should be interested, it will be given a little space. In their natural condition the great bodies of water were pure and clean. As population became more dense, they have become heavily polluted with wastes of domestic and industrial life. To-day there are few natural bodies of water that approach in any way their original condition. Fish life has been killed and they have been spoiled as well for recreational purposes.

The opinion is quite generally held that flowing streams become self-purified; students of the question like to use another term, self-improvement, since self-purification is a very strong term. The United States Public Health Service recently made a statement on this subject, part of which is given below.

Briefly, it may be stated that a water contaminated with the organic matters found in sewage and in various industrial wastes does gradually rid itself of such pollution, if allowed free access to air. Early studies of this phenomenon of self-purification led to the abandonment of a plausible theory based on the direct action of oxygen on the organic matters, and subsequent research extending over the past fifty years has revealed that the self-purification of streams is essentially a biological process. In this sense, the oxygen contained in aerated or running water does not operate as a sterilizing agent, as once believed, but rather as a neutralizing or deodorizing agent for some of the gases resulting from the bacterial decomposition of the organic matters. Dissolved oxygen is also required for the maintenance of fish life. While thus relegated to a secondary rôle, the amount and rate of disappearance of the oxygen which is contained in a given water nevertheless serves as an excellent indicator, first of the threatened disappearance of fish life and, with increasing pollution, as a warning of impending nuisance conditions. With the understanding that a bacteriological examination is a much better index of wholesomeness or fitness for drinking purposes, it has accordingly become customary to express the pollution of a given water in terms of its demand for dissolved oxygen when reference is made to the threatened disappearance of fish life or to the approach of nuisance conditions.

One of the most generally known cases of stream pollution is that of the Illinois River which is made to carry the sewage of Chicago. This situation is especially interesting on account of the litigation which took place in 1901 between the State of Missouri and the City of St. Louis *vs.* the State of Illinois and City of Chicago. The complainants claimed that their water supply would be endangered if Chicago were permitted to empty her sewage into the Illinois River. Another fact making this situation of special interest is that chemical and bacteriological analyses have been made almost constantly on the river and there is probably

no other stream in the world on the sanitary condition of which so many data are available. The situation is briefly this: Chicago, in order to lessen the pollution of Lake Michigan with sewage, pumped her sewage into the Des Plaines River which in turn flowed into the Illinois River. In order to help the situation, water from Lake Michigan was used for diluting the sewage. So much of this has been taken that now complaints are being offered that the lake level is being lowered. We have no space for a detailed discussion of all the phases of the controversies; those which have been presented should stimulate people especially interested or concerned to read into the subject.

Sewage Farming.—It was soon realized that sewage contained much food material for plants and attempts were made to utilize them on



FIG. 113.—Showing How the Sewage is Delivered to a Sprinkling Filter.
(Columbus, Ohio.)

sewage farms. These farms require a sandy porous soil and one which will not become clogged, a condition often described as "sewage sick." While at one time the method was rather widely used, it has slowly gone out of common usage on account of its real disadvantages. The farms are said to be in use about Berlin and several other cities. It is obvious that human foods should not be raised on such farms. One writer stated that much of the typhoid fever in Paris during a recent summer was due to radishes raised on sewage farms. Certain types of grass have been successfully grown, however, on these farms.

Filtration.—Two kinds of sewage filtration are used. One involves the use of trickling filters and the other intermittent filters. Both types aim at as rapid oxidation as possible of the chemical constituents in the sewage.

Intermittent Filtration: The effluent from some of the anaerobic methods may also be filtered through sand. The sand must be coarse.

The difficulties of the operation and also the scarcity of the proper sand caused the introduction of contact beds. The sewage is applied to the beds very carefully in order that they may not become clogged. The reactions which are sought in the intermittent filter are, in general, nitrification, the oxidation of reduced nitrogen to oxidized nitrogen, and similar changes. These take place mainly when the filters are empty.

Contact Beds: These are concrete basins which are filled with broken brick, coke, stone, etc., all of which greatly increase the surface area in the filter. The beds are filled, allowed to stand full for a period, emptied and allowed to "rest" for a period. The period is known as a cycle. Each of the pieces of filler becomes surrounded with a gelatinous film which during the period when the tank is full, adsorbs organic matter from the sewage. When the tank is empty, the air permeates the filter and the organic matter is oxidized.

Sprinkling Filters: In this method the hydrolyzed sewage from the anaerobic method is sprinkled periodically onto a bed filled with crushed rock. The filters are constructed of such size that while one-half of the filter is receiving sewage the other half is draining and being reaerated. The changes in the sprinkling filter are also oxidative in nature.

Aeration: Since one of the main objects in sewage disposal is oxida-

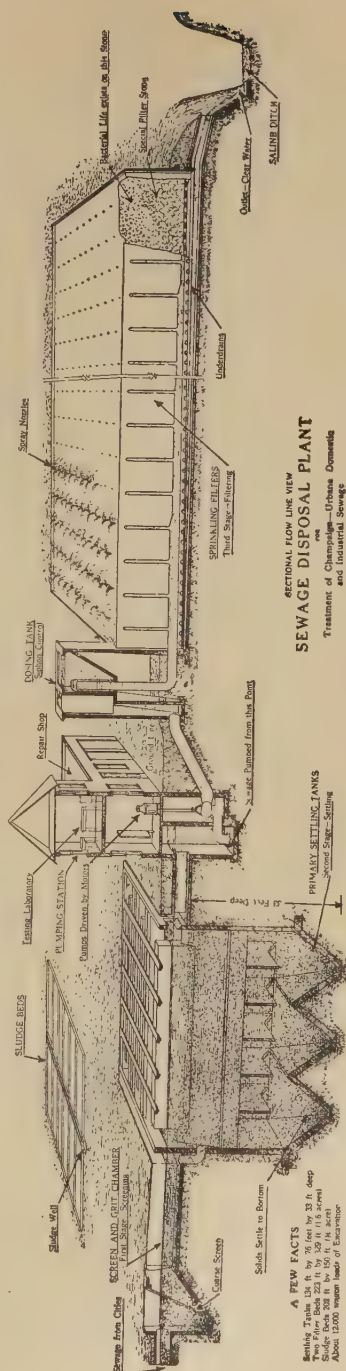


Fig. 114.—Showing the Sewage Disposal Plant. (Inhoff Tanks and Sprinkling Filters at Urbana, Illinois.)

tion, engineers have discovered that sewage may be aerated with a very rapid oxidation of reduced substances. The ammonia is rapidly and completely changed to nitrates. Under these conditions one would expect a rapid replacement of reduced compounds with oxidized compounds. It was shown by some of the early work that a tank of sewage was more rapidly oxidized if it were seeded with a little sludge from a tank which had been aerated. This indicated that something existed in this sludge which hastened oxidation. On account of this fact, such

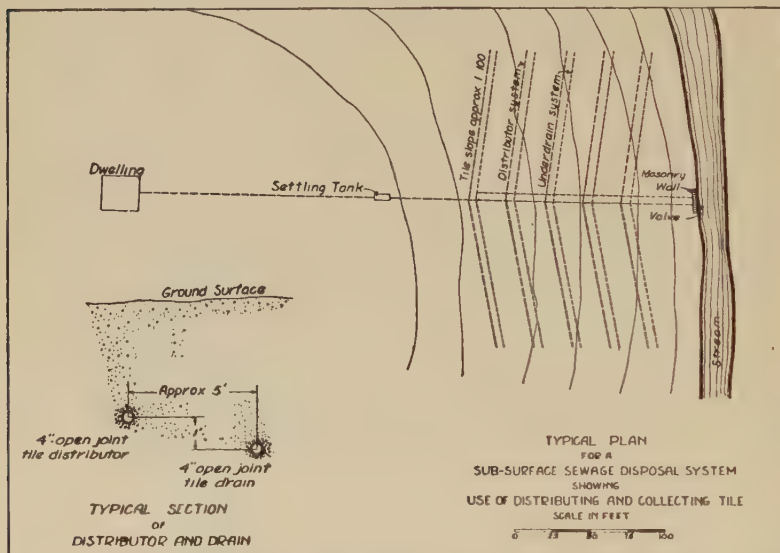


FIG. 115.—Typical Plan for a Sub-Surface Sewage Disposal System. (Illinois State Water Survey.)

sludge became known as *activated sludge* and the process of aeration as the *activated sludge method* of sewage disposal.

Swimming Pools.—The swimming pool has become an important sanitary problem in American life. It is generally admitted that swimming pools may be responsible for serious infections of the eyes and ears. Such infections have made it necessary to disinfect pools for a number of years. Unfortunately such disinfection has often been left to uninformed individuals who so overdosed the water that marked discomfort has been caused among those who used the pools. There is no reason to-day why a swimming pool needs to be overdosed with chemicals used in disinfection. In 1926 a report was presented to the Public Health Engineering Section of the American Public Health Association

on Swimming Pools and other Public Bathing Places. This report probably embodies the best information on the design, equipment and operation of swimming pools that has been connected into one publication. Those interested in the details may consult the original report.¹

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¹ Swimming Pools and Other Public Bathing Places. American Journal of Public Health, December, 1926. Also available from the Association as a separate publication.

CHAPTER XXII

BACTERIOLOGY OF MILK AND MILK PRODUCTS

Milk constitutes one of the important foods of man. Its chemical constitution makes it susceptible to bacterial decomposition and its preservation has been the subject of much study. As a cause of disease, milk is not especially important, for millions of quarts are used daily in America without causing an undue amount of illness. There are several diseases, however, the etiologic agents of which may be disseminated in milk. One of them is tuberculosis.

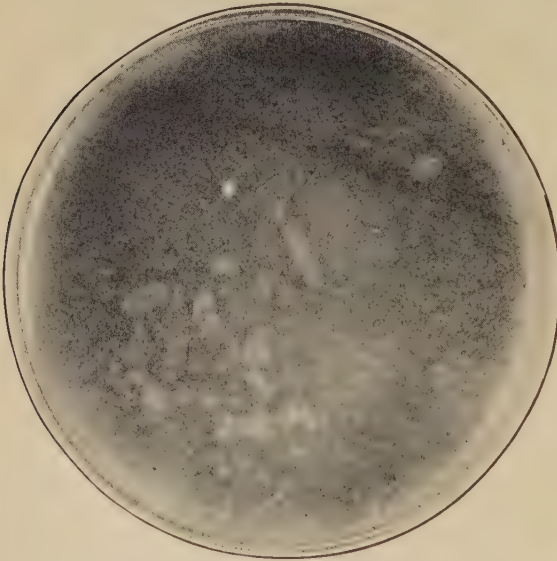


FIG. 116.—Petri Dish Preparation of Milk Collected under Clean Conditions and Properly Handled after Milking. This Milk Contained about 500 Bacteria per Cubic Centimeter. (After Magruder, 1910.)

There are several diseases, however, the etiologic agents of which may be disseminated in milk. One of them is tuberculosis.

The Importance of a Wholesome Milk Supply.—The characteristics of milk make it necessary that great care be exercised in its production and handling.¹ Unlike many foods milk is usually taken into the body in the uncooked state—not considering pasteurized milk as cooked milk. Moreover, it is used by almost every one

every day and if it is unwholesome there is constant danger of spreading infection. It is easy to see that a few gallons of infected milk might seed a large quantity and make it unfit for consumption. Milk

¹ Mohler, J. R., The Importance of a Wholesome Milk Supply. U. S. Dept. Agr. Bureau of Animal Industry, Circular 153, 1910.

constitutes a large part of the food of children, a portion of our population which is indifferent to quality in foods and therefore must be protected.

Bacteria in Milk.—Great numbers of bacteria are not desirable in milk. When large numbers are present, the milk sours more quickly. Milk with large numbers of bacteria may also contain more objectionable bacteria than milk with few bacteria. Consequently a low count milk is much more to be desired than one with constantly large numbers of microorganisms. Most of the bacteria that gain entrance to milk are harmless. Only a few are objectionable from the standpoint of disease. Professor Conn, one of America's first dairy bacteriologists, stated that good clean milk would have a low number of bacteria; a large number indicates the presence of dirt or such undesirable conditions of production and handling as high temperatures, lack of cooling, etc. The bacteria in milk gain entrance from many sources and, therefore, may be heterogeneous in type. It has become customary to speak of a "milk flora" consisting of the bacteria usually encountered in it.

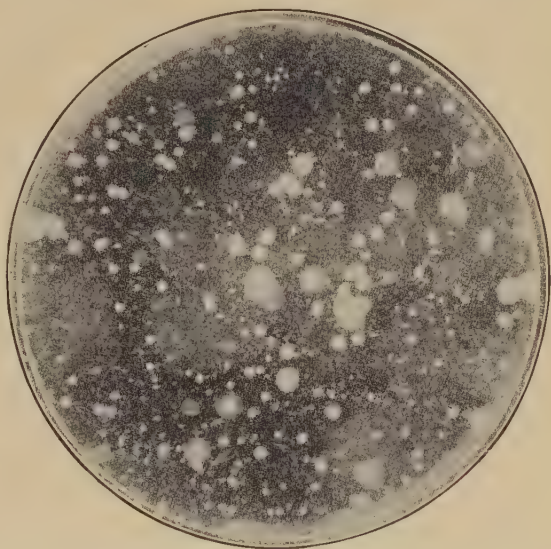


FIG. 117.—Petri Dish Preparation of Milk Not Cooled but Kept at 60° F. for Twenty-four Hours. This Milk Contained 2,800,000 Bacteria per Cubic Centimeter. (After Magruder, 1910.)

Bacteria in Milk in the Udder.—Good fresh milk is now known to contain bacteria when it is excreted from the udder. It was once believed that such milk was sterile, but recent work by one investigator resulted in the knowledge that fresh milk, when it is excreted, may contain as many as 500 bacteria per cc. These bacteria are usually of no sanitary significance, especially if the udder of the cow is not diseased. Mastitis, an inflammatory infection is often caused by streptococci. The streptococci with the pus which they cause may be excreted in the

milk. Some outbreaks of septic sore throat have been traced to the presence of streptococci from infected udders.

Factors Influencing Bacteria in Milk.—Recent experimental work has indicated that the cow's environment (barn conditions) has less influence on the bacteria in milk than was formerly supposed. Great attention was given to improvements in barn sanitation and construction and although they were greatly to be desired they did not influence greatly the numbers of bacteria in the milk. This situation made it necessary to carry out expensive research work to find out the important factors so that attention could be given to them. The utensils were found to be the most important agents in this connection. When they were carefully cleaned the number of bacteria that gained entrance to the milk was low even though the milk was produced under dirty barn conditions. Temperature influences the development of bacteria after they have gained entrance to the milk. The ideal milk is one which comes from healthy animals, is handled by healthy men and kept cold until it is delivered. It is far easier to state these requirements than it is to devise methods for determining them. For the production of wholesome milk it is necessary to have the constant cooperation of the farmer, the carrier, the distributor and the housewife.

Methods for Improving Milk Quality.—The control and improvement of milk supplies has made necessary the creation of many bureaus and departments in federal and state governments. These are concerned with laboratory analyses as well as inspections. Some of the more important methods of controlling quality will be mentioned.

Sanitary Inspection.—This step in the maintenance of a high quality water has already been discussed. It was found that sanitary inspection often made laboratory analyses unnecessary. The same stand may be taken in milk work—a sanitary inspection might reveal conditions which would preclude the necessity of laboratory work. Sanitary inspection of dairies has centered about the use of the score card on which are embodied those essentials of sanitation regarded as necessary for the production of clean milk. The score card makes it possible to obtain a numerical rating of the dairy and to stimulate rivalry among producers.

Medical Milk Commissions and Certified Milk.—This represents another important step in the improvement of milk in America. The first medical milk commission was organized in New Jersey in 1890. The State Medical Society attempted to secure improvements in the production and sale of clean milk by appealing to the state legislature. While the need for such effort was recognized, the State of New Jersey could not, at that time, institute the work. Consequently the medical

DETAILED SCORE

Equipment.	SCORE.		Methods.	SCORE.	
	Perfect.	Allowed.		Perfect.	Allowed.
COWS					
Health.....			Cleanliness of cows.....	8	
Apparently in good health.....	6		STABLES		
6			Cleanliness of stables.....	6	
If tested with tuberculin			Floor.....	2	
once a year and no tu-			Walls.....	1	
berculosis is found, or			Ceiling and ledges.....	1	
if tested once in six			Mangers and partitions.....	1	
months and all reacting			Windows.....	1	
animals removed.....	5		Stable air at milking time.....	6	
(If tested only once a year			Freedom from dust....	3	
and reacting animals found			Freedom from odors....	3	
and removed, 2.)			Barnyard clean and well		
Comfort.....	2		drained.....	2	
Bedding.....	1		Removal of manure daily		
Temperature of stable.....	1		to field or proper pit....	2	
Food (clean and wholesome)	2		(To 50 ft. from stable, 1.)		
Water.....	2		MILK ROOM		
Clean and fresh.....	1		Cleanliness of milk room...	3	
Convenient and abundant			UTENSILS AND		
.....	1		MILKING		
STABLES			Care and cleanliness of uten-		
Location of stable.....	2		sils.....	8	
Well drained.....	1		Thoroughly washed.....	2	
Free from contaminating			Sterilized in live steam		
surroundings.....	1		for thirty minutes....	3	
Construction of stable.....	4		(Placed over steam jet, or		
Tight, sound floor and			thoroughly scalded with		
proper gutter.....	2		boiling water, 2.)		
Smooth, tight walls and			Inverted in pure air....	3	
ceiling.....	1		Cleanliness of milking.....	9	
Proper stall, tie, and man-			Clean, dry hands.....	3	
ger.....	1		Udders washed and dried		
Means of light: 4 sq.ft. of				6	
glass per cow.....	4		(Udders cleaned with		
(Three sq.ft., 3; 2 sq.ft.,			moist cloth, 4; cleaned with		
2; 1 sq.ft., 1. Deduct for			dry cloth at least fifteen min-		
uneven distribution.)			utes before milking, 1.)		
Ventilation: Automatic sys-			HANDLING THE MILK		
tem.....	3		Cleanliness of attendants in		
Adjustable windows.....	1		milk room.....	1	
Cubic feet of space for cow:			Milk removed immediately		
500 to 1000 ft., 1; less than	3		from stable.....	2	
500 ft., 2;			Prompt cooling (cooled im-		
less than 400 ft., 1; less than			mediately after milking		
300 ft., 0; over 1000 ft., 0.)			each cow).....	2	
UTENSILS			Efficient cooling: below 50°		
Construction and condition			F.....	5	
of utensils.....	1		(51° to 55°, 4; 56° to 60°, 2.)		
Water for cleaning.....	1		Storage: below 50° F.....	3	
(Clean, convenient, and			(51° to 55°, 2; 56° to 60°, 1.)		
abundant.)			Transportation: iced in sum-		
Small-top milking pail....	3		mer.....	3	
Facilities for hot water or			(For jacket or wet blan-		
steam.....	1		ket, allow 2; dry blanket or		
(Should be in milk house			covered wagon, 1.)		
not in kitchen.)					
Milk cooler.....	1				
Clean milking suits.....	1				
MILK ROOM					
Location of milk room....	2				
Free from contaminating					
surroundings.....	1				
Convenient.....	1				
Construction of milk room.	2				
Floor, walls, and ceiling, 1					
Light, ventilation, screens					
.....	1				
Total.....	40		Total.....	60	

Equipment..... + Methods..... = Final score.

NOTE 1.—If any filthy condition is found, particularly dirty utensils, the total score shall be limited to 49.

NOTE 2.—If the water is exposed to dangerous contamination, or there is evidence of the presence of a dangerous disease in animals or attendants, the score shall be 0.

men did the next best thing; they suggested the formation of a commission to be composed of prominent health officials, and scientists who had established reputations in the study of milk and dairy products. The commission was to draw up a set of rules and a sanitary code under which high-grade milk was to be produced. It was believed that the report of this commission would have great weight on account of the standing of its members. Those who were invited to membership on the commission chose the name Medical Milk Commission. From this first Medical Milk Commission have developed many more scattered all over this country.

After establishing the Medical Milk Commission it was necessary to have some means of designating milk which was produced under the supervision of a Medical Milk Commission and the term *certified* was registered in the U. S. Patent Office; this was done in order to prevent the term from being degraded by dairymen having no connection with a Medical Milk Commission.² Certified milk may be defined as milk produced under a legal contract between a Medical Milk Commission and a dairyman; it must therefore meet the requirements of the commission. We need not take space to enumerate all of the requirements which must be met in the production of certified milk. Nothing is left undone (with the exception of pasteurization) which would raise the quality. Three types of examination are made. A chemist and bacteriologist makes periodic examinations of the milk, samples of which he collects himself. A qualified veterinarian examines the cows periodically. And lastly, representatives of the Medical Milk Commission also inspect the dairy. When these examinations and inspections indicate that the milk is free from disease bacteria, has a low bacterial count, possesses the proper chemical constitution and is free from foreign matter, etc., it is certified by the Medical Milk Commission. While these are very desirable requirements, it is not easy to determine them. For instance, can even a qualified veterinarian positively say that a cow is free from disease? Can a dairyman, for instance, always be assured that his hired hands are free from communicable diseases? They may be examined by physicians but develop disease immediately after the examination or they may be infected and the infection may have been overlooked. Some of the help may regard a slight sore throat as of negligible importance and say nothing about it. It will be apparent after a little thought that while the conditions for the production of certified milk are reasonable and desirable, there may be opportunities for undesirable milk to be certified. This is proven by the several out-

² Lane, C. B., Medical Milk Commissions and the Production of Certified Milk in the United States. U. S. Dept. Agri., Bur. of Animal Industry, Bull. 104, 1908.

breaks of communicable disease which have been traced to certified milk. Such milk is not necessarily safe milk, although in most cases it probably is. Properly pasteurized milk is safe milk.

It is well to point out definitely that certified milk is not the result of effort on the part of any government bureau. As stated in one of the pamphlets of the American Association of Medical Commissions, "the standards of cleanliness which govern the production of all certified milk were established by the medical profession." The originators of certified milk were a group of doctors who realized the need for a milk so pure and delivered so fresh that it could be used untreated exactly as nature intended all milk should be." Those interested in the production and use of certified milk have united to form The Certified Milk Producers' Association of America and the American Association of Medical Milk Commissions.

Pasteurization of Milk.—Pasteurization of milk consists in heating it to a certain temperature, holding it at that temperature for a period of time, and cooling it promptly. Health authorities are agreed that this is the most practical, economical, positive method for preventing disease dissemination by milk. Pasteurization of milk has been studied for thirty years and scientists have about agreed that a temperature of 142° F. (61.5° C.) with a holding period of thirty minutes will render milk free from disease-producing bacteria. The question will probably arise, why did bacteriologists select just this time and this temperature from many other possible combinations? It has been found that *Mycobacterium tuberculosis* is one of the important disease-producing bacteria in milk and that it is as resistant as the other common pathogenic types, if not more so. Consequently experimental work has centered about this microorganism. It has been found that the above-mentioned temperature and time will destroy the tubercle organism in milk. This statement of course implies that the milk will be thoroughly heated and held for the full holding period.



FIG. 118.—Cap from a Bottle of Certified Milk.

Pasteurization when properly carried out is one of the simplest means of making milk safe. Improperly pasteurized milk gives the consumer a false sense of security. Some states have created bureaus for controlling pasteurization plants and inspecting pasteurizing equipment. This will make pasteurization a still safer procedure. Finally, milk after pasteurization must be handled carefully and protected from contamination.

Pasteurization has made great strides in the United States. Of all milk sold in cities with a population of 500,000 or over, 98 per cent is

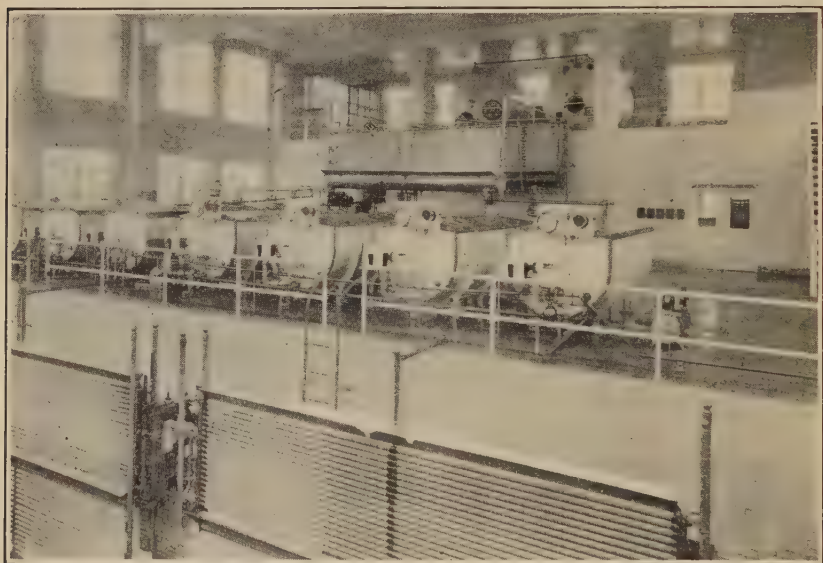


FIG. 119.—Showing the Pasteurizing Room of the Highland Dairy Farms Co., St. Louis, Mo. (Courtesy of the J. G. Cherry Co., Cedar Rapids, Iowa.)

These machines are automatically controlled so that the milk is heated in accordance with the best practice.

pasteurized. The tendency in the state of Illinois is toward pasteurization of all milk which is not certified or produced under conditions equivalent to those enforced for certified milk. The City of Chicago requires the pasteurization of all milk which is not certified.

Methods Used for Determining Quality in Milk.—No one would deny the desirability of milk of high quality and safety. Much discussion has arisen, however, over the methods that shall be used to determine this condition. The details of these procedures need not concern students who are beginning the study of bacteriology. We have just

considered some of the methods for improving the milk supply as a whole. The best methods for determining quality are, probably, laboratory methods. These involve determining the numbers of bacteria which are present and, in some cases, the types. Such methods are usually not available to the consumer. The dirt test may be applied with little apparatus. It consists in examining the bottom of the bottle immediately after delivery and before the bottle has been shaken. A little dirt will be found in all milk, even that produced under the best of conditions. A great amount of dirt is objectionable and indicates unsatisfactory methods of production and handling. In the laboratory a pint of milk is forced through a cotton disk which holds back the dirt. This disk may then be preserved for examination and comparison.

Grading of Milk.—On account of various methods of producing milk and different degrees of interest in quality, it is useful to have a system for grading milk. Among many is that proposed by a Committee of the American Public Health Association, as follows:

The Committee begs to report as follows: 1. *Resolved* that "All milk should be classified by dividing it into three grades which shall be designated by the letters of the alphabet." The requirements for the three grades should be as follows:

Grade A

2. Raw Milk.—Milk of this class should come from cows free from disease as determined by tuberculin tests and physical examinations by a qualified veterinarian, and should be produced and handled by employees free from disease as determined by medical inspection of a qualified physician, under sanitary conditions, such that the bacterial count does not exceed 10,000 per cc. at the time of delivery to the customer. It is recommended that dairies from which this supply is obtained score at least 80 on the United States Bureau of Animal Industry score card.

3. Pasteurized Milk.—Milk of this class should come from cows free from disease as determined by physical examinations by a qualified veterinarian, and be produced and handled under sanitary conditions such that the bacteria count at no time exceeds 200,000 per cc. All the milk of this class should be pasteurized under official supervision, and the bacterial count should not exceed 10,000 per cc. at the time of delivery to the consumer. It is recommended that dairies from which this supply is obtained score at least 65 on the United States Bureau of Animal Industry score card.

Grade B

4. Milk of this class should come from cows free from disease as determined by physical examinations, of which one each year be by a qualified veterinarian and should be produced and handled under sanitary conditions

such that the bacteria count at no time exceeds 1,000,000 per cc. All milk of this class should be pasteurized under official supervision, and the bacterial count should not exceed 50,000 per cc. when delivered to the consumer.

It is recommended that dairies producing Grade B milk should be scored and that the health departments or the controlling departments, whatever that may be, strive to bring these sources up as rapidly as possible.

Grade C

5. Milk of this class should come from cows free from disease as determined by physical examinations, and should include all milk that is produced under conditions such that the bacterial count is in excess of 1,000,000 per cc.

All milk of this class should be pasteurized, or heated to a higher temperature, and should contain less than 50,000 bacteria per cc. when delivered to the consumer.

6. Whenever any large city or community finds it necessary, on account of the length of the haul or other peculiar conditions, to allow the sale of Grade C milk, its sale should be surrounded by safeguards such as to insure the restriction of its use to cooking and manufacturing purposes.

Relation of Milk to the Spread of Disease.—The fact that milk may spread disease has caused it to receive the attention of public health officials. While milk has been said to disseminate infant paralysis, scarlet fever, septic sore throat, etc., the best known disease is, perhaps, tuberculosis. This disease attacks the cow and the tuberculosis microorganisms may appear in the milk. It is now generally admitted that the bovine type of the tuberculosis organism may cause infection in human beings. It has been stated that about one-tenth of the tuberculosis of bones, joints and lymph nodes in adults and one-fourth of tuberculosis of these types in children is due to the bovine type of the microorganism. About 10 per cent of the tuberculosis in human beings below the age of ten years is due to the bovine type of the microorganism spread by milk. One is quite liable to consider such data indifferently until he has an opportunity to study data on definite cases. The report of one case may be reproduced here. It is taken from one of the dairy journals.

————— of Edgar County had his cattle tested for tuberculosis and one cow reacted. He did not believe in the tuberculin test and later took the tag out of the animal's ear. The cow looked healthy and he told his men that there was nothing the matter with her. He talked so much that one of his tenants was induced to take her in payment for work. He used her for a family cow. In his family there were seven children. In the spring some of the children commenced to complain and were sickly. Finally a physician was consulted and five of the children were found to have tuberculosis. The oldest and youngest children did not take the disease. The youngest child was still nursing and did not use the cow's milk. The oldest child was not allowed to use the cow's milk since it was thought better to give it to the

younger children. The five children were sent to a tuberculosis sanitarium. It was thought that one of them would die. The farmer was fined \$150 for selling a tuberculous animal. The cow was killed and when posted, showed generalized tuberculosis.

Another account quite similar to the one just reported was published by King in the Journal of Veterinary Medical Association. The details in this account are just as convincing. Correspondence with Dr. King revealed that other cases had been found; he believed that it was possible to find them in almost any community if any one would take the time to search for them. Such accounts should convince anyone of the value of pasteurization; they should stimulate us to take an active part in all effort to stamp out disease.

Tuberculosis in cattle is a deceptive disease because its symptoms are not striking. The disease progresses insidiously, during which time virulent tubercle bacilli may be excreted in the milk. The United States Department of Agriculture has stated that the loss of cattle each year from tuberculosis amounts of \$40,000,000 in the United States.

Typhoid fever is also a milk-disseminated disease. It differs from tuberculosis in that the etiologic agent is not contributed to the milk by the cow. Milk becomes contaminated with *Eberthella typhi* (*Bacterium typhosum*) as do other foods. The infection may be carrier-borne. The typhoid organisms may also multiply in the milk. Frost enumerated the following factors which influence the dissemination of typhoid fever by milk.

1. The sources of infection to which milk is exposed.
2. The opportunities afforded for infective material to be introduced into milk from these sources as well as the precautions taken to safeguard against the introduction of infective material.
3. Circumstances affecting the potentiality of the milk supply in disseminating infection after infective material has once been introduced.

The most important sources of typhoid bacilli in milk are human discharges. The possibility of contamination with human discharges increases with the number of people who handle the milk. Those individuals with a known history of typhoid fever ought not to handle milk until they have been shown to be free from danger of excreting the bacilli.

BUTTER

Butter is a food the flavor of which depends to a great extent on bacterial activity. The butter maker desires an abundant growth of those bacteria which will improve the flavor of his product. The bacteria play their greatest rôle in this connection in cream ripening.

The cream is pasteurized after separation from the milk, to reduce as far as possible the extraneous types of bacteria, or those forms which might harm the flavor of the butter. The cream is then inoculated with a starter, or culture of desirable lactic acid bacteria, that grow in the cream and form products which bestow the desired flavor. The use of such a starter helps to standardize the flavor of the butter from batch to batch, makes churning easier, and gives a butter with better keeping qualities. The use of such a starter gives what is called a sour cream butter. Sweet cream butters have a milder flavor and are made from cream which has not soured. The desire in the United States for a butter with a more marked flavor has led to the manufacture of sour cream butter almost entirely.

The bacterial content of the finished butter is of little consequence so long as the bacteria are non-pathogenic. Fresh butter may contain as many as 50,000,000 bacteria per gram. This number rapidly decreases until after a month or two only a few hundred thousand are present.

In discussing the bacteriology of milk *Mycobacterium tuberculosis* was mentioned as one of the important pathogenic microorganisms which might be present in milk. Consequently, if butter is made from tuberculous milk, the tuberculosis organisms will be found in the butter. Experiments have been carried out to determine how long tuberculosis organisms could live in butter. In one of these experiments cream was inoculated with tubercle bacilli and then churned into butter. After 133 days tubercle bacille were demonstrable in the butter, indicating that salting and storing butter did not destroy tubercle bacilli. *Eberthella typhi* (*Bacterium typhosum*), causing typhoid fever, has also been isolated from butter after long storage. Consequently, to have safe butter uninfected ingredients must be used for its manufacture.

CHEESE

Cheese is an important dairy product both from the viewpoint of the dairy industry as well as of human food. Many different kinds are made depending on the microorganism used for ripening and other factors. In the chapter on Molds two kinds of cheese were described which were ripened by molds. Bacteria are used in the preparation of many different kinds. Cheeses are roughly divided into two groups, soft cheeses and hard cheeses. Cheese is, in general, prepared by taking the curd of the milk, salting it and subjecting it to a period of ripening. The ripening process causes the development of that flavor for which the cheese in question is known. During the ripening period the microorganisms which were added to the curd grow and form compounds

which impart the characteristic flavor to the cheese. Cheese ripening is, then, a hydrolysis of the proteins in the curd. Some bacteria are undesirable in cheese. They bring about the development of undesirable flavors, softening, bad odors, etc. In a few instances poisonous products have been reported.

Cheese, along with other dairy products, may contain pathogenic bacteria. We have learned that tuberculosis is one of the common diseases disseminated by dairy products. The tubercle bacilli may be spread by cheese also. One investigator made cheese from milk which had been inoculated with tubercle bacilli and found the organisms to be alive after 104 days' storage. This is further evidence for using care in selecting our foods and justifying the widespread agitation over disease-disseminating dairy products. It might be of interest to report some experiments on the examination of ordinary market cheese for tubercle bacilli. Two investigators of the United States Department of Agriculture examined 256 samples of cheese purchased on the open market in Washington, D. C. Nineteen or 7.42 per cent caused tuberculosis in guinea pigs when injected according to the usual bacteriological technic. These investigators stated, however, that there was little danger in eating ripened cheese because most of the tubercle bacilli were killed during the ripening process. Cream cheese was found to be especially liable to cause tuberculosis since, when the milk is centrifugalized, the bacilli appear in the cream. This indicates that milk used for the making of cheese should be pasteurized.

Different Types of Cheese.—There are many different types of cheese distinguished from one another in different ways. Some cheeses are made from curd separated from the milk with acids; these are called acid-curd cheeses. Others are made from curd separated by rennin; these are called rennin curd cheeses. Some are named from the town or locality in which they were originated.

Cheddar Cheese.—This cheese is the common hard variety which is sold in America. It is made in different countries but the greatest amount is made and used in America. Cheddar cheese is made by curdling the milk with rennin and cutting it into small pieces after which it is salted and pressed into large cakes or heads. The bacteria continue to grow and produce decomposition products which are characteristic for this type.

Camembert and Roquefort Cheese.—These types are discussed in the chapter on Molds.

Emmentaler Cheese.—This is also known as Swiss cheese. It is characterized by the appearance of numerous large holes containing gases formed in the fermentation of the (lactose or milk sugar). The gas is mainly carbon dioxide; propionic and acetic acids are also formed in appreciable amounts.

Limburger Cheese.—Limburger cheese is a putrefactive product. It is prepared by allowing the decompositions which take place in the making of practically all cheeses to go further toward completion. Instead of the proteins being left in the peptone and proteose stages, they are taken to amino acids and other decomposition products which have been discussed in the chapter on the Nitrogen Cycle. As stated above all cheeses are really putrefactive products, the flavor or aroma depending on the special decomposition products which are formed.

ICE CREAM

This dairy product enjoys great popularity. The possible undesirable conditions that may obtain in other dairy products, some of which have just been discussed stimulated investigations concerning ice cream. The bacterial count of ice cream may be high and depends on the bacterial content of the several ingredients from which the ice cream is made. While many investigations of the bacterial content of ice cream have been reported, most of them are in fair agreement. Ayers and Johnson studied ice cream purchased on the open market in Washington, D. C., in 1912 and 1913. Their data may be briefly summarized as follows:

Number of Samples	Maximum Number of Bacteria	Minimum Number of Bacteria	Average Number of Bacteria
94 Summer samples.....	510,000,000	120,000	37,859,907
91 Winter samples.....	114,000,000	13,000	10,388,000

It is apparent that winter ice cream has a much lower bacterial content than summer ice cream. This is probably best explained by the fact that the various ingredients used in the manufacture of ice cream are more easily kept during the winter.

Ice cream has also been incriminated in disease dissemination, but it is probably no more important than the other dairy products. Outbreaks of typhoid fever have been traced to ice cream prepared by carriers. The danger, however, is relatively insignificant.

FERMENTED MILKS

In many countries where herds of lactating animals constitute their owners' wealth as well as main source of food, it is necessary to attempt preservation of the milk. The best method of preservation is souring, in which condition the milk will keep for a long period of time. In

America milk is soured for therapeutic use; such milk is spoken of as fermented milk. These fermented milks vary from the by-product of butter making, buttermilk, to milks fermented with pure cultures of bacteria. In the countries of southern Europe where soured milks are used to a considerable extent, many kinds are made depending on whether the milk from the mare, sheep, goat, etc., is used as well as upon the microorganism used for the fermentation. It is not necessary to discuss all of these types.

The use of soured milks in the diet is an old practice but the therapeutic use of these milks is, perhaps, of newer interest, at least in America. Metschnikoff once explained longevity of human beings, especially those living in southern Europe, on the ground that they used large amounts of fermented milks. Premature death, according to Metschnikoff, was due to arteriosclerosis (hardening of the arteries); this, in turn, was caused by autointoxication; autointoxication resulted from intestinal putrefaction and intestinal purefaction from the presence of undue numbers of putrefactive bacteria in the intestines. Metschnikoff argued that if these putrefactive bacteria could be replaced by saccharolytic bacteria the evil products of putrefaction would not be formed to be taken into the blood. Therefore, he advised the use of fermented milk which contained the lactic acid bacteria; these split carbohydrates and replaced those which decomposed protein. It is well known that the putrefactive bacteria cannot tolerate the presence of bacteria which split carbohydrates because the latter form organic acids which are inimical to the putrefactive bacteria.³

Metschnikoff also believed that these undesirable bacteria could be replaced by a process called "implantation"; the desirable acid formers were believed to take up their abode in the human intestines to act as continual inhabitants. The organism which was believed to do this best was *Lactobacillus bulgaricus* (*Bacillus bulgaricus*). These claims of Metschnikoff were not without study. Numerous investigators tried to confirm Metschnikoff's statements. In America a series of investigations were instituted at Yale University under the direction of Professor Rettger. These experiments were, in the main, concerned with an organism called *Lactobacillus acidophilus* a very near relative of *Lactobacillus bulgaricus* and probably confused with it by many of the earlier workers.

Milk fermented with *Lactobacillus acidophilus* is known under the name of "acidophilus milk." This fermented milk has been much advised for many different ailments; it seems to be of greatest value when

³ How many other instances of this can be mentioned?

undesirable conditions exist in the alimentary tract. Rettger found that a carbohydrate rich diet along with a heavy inoculation of the bacilli would bring about desirable changes in the shortest time. It is generally agreed that the daily ingestion of sufficiently large amounts of lactose results in a marked increase in the numbers of *Lactobacillus acidophilus*, since they are always present in small numbers; in due course of time other intestinal bacteria are reduced to a negligible number. Bass found, however, that it required more than 300 grams of lactose a day to establish *Lactobacillus acidophilus* as the predominating microorganism. Unfortunately such quantities of sugar are too large to be taken over a long period of time; hence the practical value of this method is quite limited. The investigations made by Bass on the use of cultures of *Lactobacillus acidophilus* for therapeutic purposes, including a large number of experiments on twenty-three human subjects, have confirmed the statements of Rettger and his colleagues. Bass called attention to the danger of making the same kind of mistake with *Lactobacillus acidophilus* that was made with *Lactobacillus bulgaricus*. He stated that enthusiastic workers have reported most striking therapeutic results with the administration of broth cultures of *Lactobacillus acidophilus* in teaspoonful doses, only a small fraction of the amount of culture that others have found necessary to change noticeably, the intestinal flora.

The market is flooded with many preparations including acidophilus milk, tablet and liquid cultures of the organism. Bass examined some of these preparations to determine the number of viable cells therein. In the tablets he could never find more than 1000 viable cells per tablet. If it should be granted that all of these 1000 viable cells were *Lactobacillus acidophilus*, it would take more than 1,000,000,000 tablets, or more than 20 tons, to contain as many living bacilli as are contained in 1000 cc., the ordinary daily dose, of acidophilus milk, the quantity found by most investigators to be necessary for the transformation of the flora. Bacteria were somewhat more numerous in the liquid cultures. If all of the viable cells in the liquid cultures were *Lactobacillus acidophilus*, a patient would have to drink 7 or 8 gallons daily to get as many as he would secure in 1000 cc. of properly prepared, fresh acidophilus milk. Such information indicates the insidious properties of some of these preparations.

The work of Bass, quoted above, is nicely confirmed by a report by James which appeared very recently. James examined 107 samples of commercial acidophilus preparations of which thirteen produced in reasonably pure form the species of microorganism indicated on the label; fifteen of the remaining samples were sufficiently pure and pre-

sented viable organisms in sufficient numbers to have possible value. The rest were practically worthless as representing cultures of the species indicated.'

CONCENTRATED MILKS

There are several types of concentrated milks on the American market. Of these the condensed and the evaporated are best known, although milk powder is rapidly gaining a place. In the preparation of condensed and of evaporated milks much of the water is driven off in vacuum pans. Sugar is added to the condensed milk while the evaporated milk is unsugared. The sugar helps to preserve the condensed milk. Most of such milk is canned for distribution to the public. Some is held under aseptic conditions for the making of ice cream. Bacteriological examinations of cans of condensed and evaporated milks have shown that they may not be sterile. The bacteria may be dormant and not develop. Such bacteria are of no significance. Bacteria which can grow in the cans and cause spoilage usually do so before the cans reach the consumer.

Milk powder is a more recent type of concentrated milk. It differs from condensed and evaporated in that most of the water has been removed. Milk powder is not sterile but may contain varying numbers of bacteria. Some sanitarians have stated that dried milk would eventually replace the present supply of fresh milk.

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CHAPTER XXIII

INDUSTRIAL FERMENTATIONS: USE OF BACTERIA FOR THE PREPARATION OF VARIOUS SUBSTANCES

At several places in this book mention has been made of useful application of microorganisms for carrying out certain procedures. The bacteria are best known by the layman at least, in their disease-causing relation. In this chapter instances will be discussed where they play a useful rôle in helping man to prepare certain chemicals which would be made only with difficulty by other methods. These are often spoken of as biological or industrial fermentations. Lack of space compels the selection of a few of the more common and important ones for discussion.

VINEGAR FERMENTATION

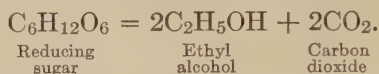
The word vinegar comes from the French words meaning sour wine. This indicates one of the first raw materials which was used for making vinegar. The microbiology of the vinegar fermentation was one of the early subjects for study. Pasteur helped to draw attention to this product by a classic report which still has value for present-day bacteriologists.

Vinegar is probably not a food in the sense in which many other substances are. It is a fermented product containing over 4 per cent of acetic acid along with other substances which give it the proper color, flavor and aroma. It is used both as a condiment and a preservative.

Raw Materials for Vinegar Making.—Any material which has an appreciable carbohydrate content is suitable for the making of vinegar. The most common raw material in America is probably the expressed juice of the apple or pear. Grape juice is also used. Wine, as has been stated above, is a good raw material for it contains alcohol. Most wines have to be diluted in order to reduce the concentration of alcohol to a degree that can be tolerated by the vinegar bacteria. Honey and orange juice have also been used; these must first of all be subjected to the alcoholic fermentation. In general, any carbohydrate substance which is not suitable for use as it is or which is available in such large quantities that it cannot be used, may be acetified.

Chemistry and Bacteriology of Vinegar Making.—Two main chemical reactions are involved in acetification of saccharine materials for making vinegar. The first is the alcoholic fermentation; this is followed by the acetic acid fermentation in which the alcohol formed in the first step is oxidized to acetic acid.

The Alcoholic Fermentation.—The alcoholic fermentation is brought about by yeasts which change the sugar to alcohol according to the following equation:



The alcoholic fermentation may be induced by putting the fresh apple juice into large tanks or vats where it will undergo the alcoholic fermentation. This fermentation may be quickened by the addition of a pure culture of yeast. The time required for completion of the alcoholic fermentation depends on various factors such as the activity of the yeasts, the temperature, the amount of sugar present, etc. At the close of the alcoholic fermentation, the second part of the vinegar fermentation must take place. For each 100 parts of sugar in the substrate there should be 51 parts of alcohol. This is the theoretical amount; it is a little more than the amount which is usually secured, for the yeast uses a little for its own purposes.

The Acetic Acid Fermentation.—Having changed the sugar to alcohol, it is necessary to change the alcohol to acetic acid. This is spoken of as the acetic acid fermentation or as acetification. It is an oxidation reaction taking place according to the following equation:



The organisms bringing about this change are bacteria grouped physiologically under the name "acetic acid bacteria." They belong to the genus *Acetobacter*. These bacteria require special conditions in which to do the greatest amount of work. The material which they ferment should not contain more than 15 per cent of alcohol, preferably less than 10 per cent. For each 100 parts of alcohol there should be 130 parts of acetic acid in the vinegar.

Acetic Acid Bacteria (Acetobacter).—Pasteur wrote an early paper on vinegar fermentation but it remained for Emil Christian Hansen, a Danish bacteriologist, to reveal the more deeply lying facts. Hansen described two microorganisms under the names *Bacterium aceti* and *Bacterium Pasteurianum*. Another organism was described later as *Bacterium Kuntzingianum*. All of these organisms differed in salient characteristics, which need not be reproduced here, as descriptions are

available in various texts. The members of *Acetobacter* are quite pleomorphic—an observation which was made by even the earliest workers with them. Another active organism in the oxidation of alcohol to acetic acid is *Bacterium xylinum*. All of the above names have been changed to *Acetobacter aceti*, *Acetobacter xylinum*, *Acetobacter Pasteurianum*, etc., in accordance with the more recent classifications of the bacteria. Pure cultures of these bacteria have been found to give a better vinegar. They are supplied by some of the Agricultural Experiment Stations.

The organisms concerned in acetification grow on the top of the substrate in a thick jelly layer which is known as “mother of vinegar.” This is comparable to the starters which are used in the dairy industry. The “mother” floats on the surface of the acetifying liquid; it is made up of both the bacteria and yeasts which live together.

Methods of Making Vinegar.—All of the several methods of making vinegar differ mainly in respect to the mechanical devices in which the yeasts and bacteria work. They involve special contrivances for giving bacteria optimum conditions in which to live. Man has other situations quite similar to this. Bees are given specially constructed houses in which to lay away honey. The septic tank is a specially made tank in which anaerobic bacteria decompose organic matter. So the vinegar maker resorts to the same practice if he wishes to have the microorganisms do the greatest amount of work.

Domestic Method.—This is the commonest method for the preparation of vinegar under domestic conditions. The casks are usually 50-gallon barrels fitted as shown in Fig. 120. The barrel should be so filled and constructed that

there is plenty of air and an opportunity for drawing off the vinegar and adding more substrate to be fermented. The time required depends on several factors. The main disadvantage of the domestic

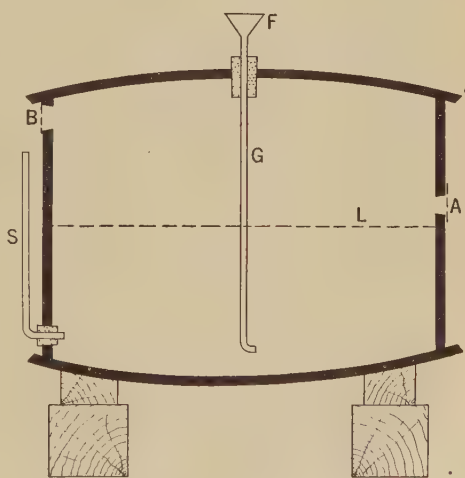


FIG. 120.—Cask for Vinegar Fermentation.
(After Bioletti.)

A, Air inlet, air passes over the surface of the medium; B, air outlet; F and G, funnel and glass tube for filling; S, tube for drawing off vinegar. A spigot may be used.

method is that it is regarded as an automatic process which requires very little attention. The use of pure cultures according

to directions which may be secured from several experiment stations will give a final product not only richer in acetic acid but possessed of a better flavor and aroma.

The Quick Vinegar Method or German Method.—In this method the vinegar maker has done what he can to help the microorganism to work at a rapid pace. The apparatus used is called a generator. It is an upright cylindrical tank filled with beechwood shavings, or similar materials. This tank is fitted with devices for allowing the alcoholic solution to trickle slowly down through the shavings on which the acetic acid bacteria are living. The tank is not allowed to fill, for that would keep out the oxygen. About the bottom of the generator are holes for allowing the air to be drawn in; the air rises through the generator and is used by the acetic acid bacteria for oxidizing the alcohol. In many respects the generator is

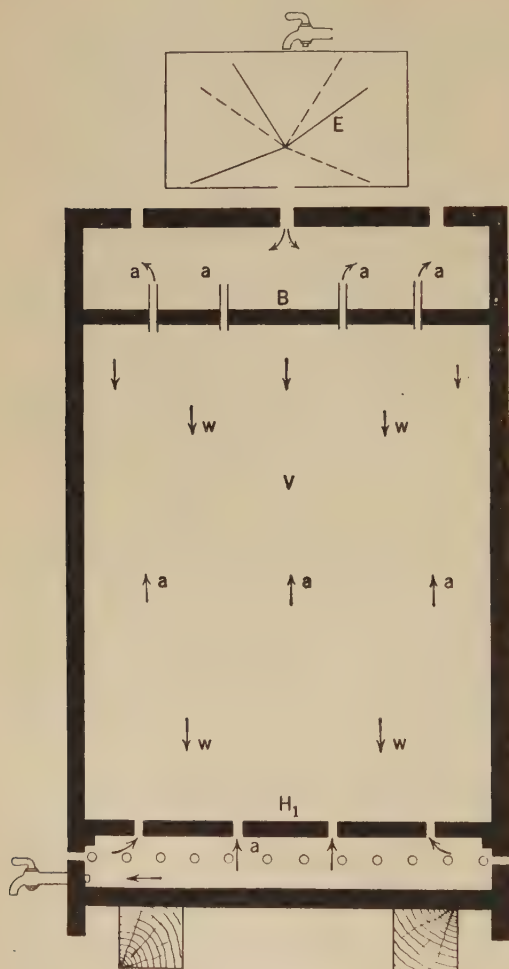


FIG. 121.—Cross-section of Vinegar Generator.
(After Bioletti.)

V, Mass of beech chips over which the alcoholic material trickles from the small holes in the flase head *H*; it becomes acetified; *E*, tilting trough for an intermitten supply of solution to be acetified; *a*, course of air; *w*, course of alcoholic solution; *t*, thermometer.

like a furnace. Both are contrivances in which active combustion takes place. Coal is added to the furnace to be chemically oxidized.

Alcohol is added to the vinegar generator to be oxidized by bacterial enzymes. Both yield large amounts of heat. In the furnace the heat is desired; in the vinegar generator too much heat must be avoided and careful watching is necessary to prevent the temperature from rising too high and destroying the bacteria.

Orleans Method.—The Orleans method is a modification of the domestic method and need scarcely be mentioned as special. It is a continuous method, new unfermented substrate being added as vinegar is drawn off.

Pasteur Method.—This was introduced to obviate some of the objections of the domestic and Orleans methods. The “mother of vinegar” is allowed to form on a wooden float. When new substrate is added the mother is thus not broken up and the fermentation is not upset.

SILAGE FERMENTATION

Certain plants which have a high water content and yet sufficient food materials to make them valuable may be preserved by storing them under conditions which prevent putrefaction but allow them to undergo a desirable fermentation. This fermentation not only preserves them but also causes an improvement in the flavor. Such materials as chopped hay, cornstalks, beet leaves, etc., are packed into a silo in which the bacteria contained in the raw materials carry on an anaerobic fermentation. Organic acids, said to be chiefly lactic, are formed; these preserve the silage in the same manner that other vegetables are preserved by fermentation. The putrefactive bacteria will not grow in the presence of acid-forming bacteria or their products. In poor silage other fermentations may take place, such as the formation of butyric acid. It is apparent, then, that the proper fermentation must be started as soon as possible in order to prevent the initiation of undesirable changes. Some work seems to indicate that the plant cells may play a rôle in silage fermentation. The important changes, however, are probably bacterial in nature. The bacteria involved are said to belong to the “lactic acid group.” Both bacilli and coccus forms have been found to be present.

TEXTILE FIBERS

For ages man has made use of certain structures in plants. They have been used for clothing, rope, and the making of materials on which to write.

Linen.—*Linum usitatissimum* has been cultivated for thousands of years as a textile fiber producing plant. The Egyptians must have

raised it, since their mummies are found to-day wrapped in fine linen. Frequent allusions to flax and linen in the Bible indicate that the ancients were acquainted with the usefulness of the bast fibers in flax and had methods of separating them from the rest of the plant. They were also familiar with other types of fibers, since they are found in their papyri to-day. The United States cannot be regarded as a great flax or linen-producing country; it has had to depend mainly on importation to supply the increasing demands for linen. Russia was once the largest flax fiber-producing country, contributing 80 per cent of the flax fiber used in making linen. Since the World War, however, this has changed on account of the industrial disorganization in that country.

Flax is raised in this country mostly for seed which is pressed for linseed oil; a smaller amount is raised for the fiber. Flax raised for seed is of a different quality from that usually required for fiber. Fiber flax is taller and produces less seed. It requires greater care in cultivation, and especially careful handling at the harvest. Some claim that it must be pulled, not cut, and tied up carefully in bundles. This may be one reason why it has been difficult to utilize the flax from seed flax for spinning. It might be possible in the future to combine profitably the seed and fiber crop. This might tend to reduce the value of each crop taken by itself, but the value of the combined crops of seed and fiber might compensate for any decrease in the value of the single crop.

The bast fibers, which are those used in making linen, are cemented to the other parts of the stalk and to each other by means of materials, for convenience, called pectins. Undoubtedly this term is used only in a general way to cover a number of compounds closely related chemically. The aim of the retting process is to remove these "binders" without harming the cellulose fiber. The fermentation must be checked when these fibers have been freed by the hydrolysis of the pectose or salts of pectic acid. These binding materials which hold the stalk together are undoubtedly carbohydrate in nature, and thus susceptible to the action of microorganisms.

The fiber is prepared from the flax straw by a special process which seems to have been built up after a long period of time without much assistance from the sciences. Proper harvesting is very important. Fiber flax should be pulled either by hand or by machinery and tied into bundles which are shocked for curing. Cutting the flax is claimed by some to leave the ends of the stalk exposed for undesirable decompositions. When the heads are shocked for curing this cut end becomes susceptible to the attacks of undesirable bacteria. The fibers become badly stained also. This may not be entirely true, however, under actual practice.

After curing, the stalks are retted. This is really a rotting process, which indicates the origin of our present term. Three general methods may be used to dissolve the binder which holds the cellulose fibers to the woody materials: dew retting, water retting, and chemical retting. The first two only are of bacteriological interest.

Dew retting was used by our forefathers in this country for preparing flax fiber for spinning and represents the earliest method of preparing flax fiber. No special apparatus is needed, since the flax straw is merely spread on the ground in the fall and allowed to remain throughout the winter. Dew retting has been used for the preparation of most Russian flax fiber. Its greatest objection is the time required, but this may be reduced greatly by carrying the process out under conditions where the retting organisms may be made to work harder.

Water retting was introduced undoubtedly to get away from certain of the distinct disadvantages of dew retting. It is carried out either in slowly flowing rivers or in ponds and other inclosed bodies of water. The bundles of flax straw are packed into these basins and weighted down. The retting process starts with a gaseous fermentation of the carbohydrate materials in the flax straw. If conditions are favorable, a little over ten days are necessary for the completion of the fermentation. The flax should be removed when all the pectic materials are dissolved, or over-retting will result. The bundles are removed, dried in sun and air, and are then ready for scutching. The river Lys in Belgium is famous for its flax retting. River retting has certain economic features which limit its wide application. As Kuhnert¹ has shown, the stream becomes putrescible, which is detrimental to fish life. It carries amounts of organic materials in the reduced conditions and may give off objectionable odors. Water retting has not had wide application in this country.

Several attempts have been made to improve on the water retting. One of the earliest of these was proposed by Schenck in 1846. The flax straw was packed tightly into a tank and the water kept at a temperature of 75° to 95° F. This warm environment was more favorable to the development of the bacteria concerned in this process, and a vigorous fermentation quickly established itself. The vats had all of the characteristics of a fermentation mixture. Others have proposed similar methods with a higher temperature.

Scutching is the process by which the woody material is broken away from the cellulose fibers after they have been retted and dried. Different methods have been used, all of which depend on breaking the

¹ Kuhnert, Landw. Wochenbl. Schleswig-Holstein. 70:540-543. 1920.

woody particles and mechanically removing them from the stalk. The fibers are finally combed to separate the "tow" from the fibers which are not long enough to remain in line. The latter may be used in paper, coarse linen, etc. The fiber from flax may be 30 to 40 inches in length, thus yielding a product which is valuable for spinning.

Different bacteria have been found which will rett flax. These microorganisms were investigated as early as 1879 when van Tieghem reported that an anaerobic bacterium named *Clostridium amylobacter* quickly decomposed the pectic substances in the flax stalk. Since that early report many other species have been found to be useful in this respect.

Hemp.—The procedures in the preparation of the textile fibers from hemp are not unlike those already described for linen. When the plants have matured they are pulled and tied into bundles. Retting is similar to the dew retting of flax for the separation of the bast fibers. The use of the fibers in hemp for the making of textiles and rope goes back to ancient times. Frequent references were made to its use by the ancient writers.

Deterioration of Textile Fibers by Bacteria.—Since textile fibers are frequently cellulose in nature, and since there are numerous bacteria which are able to decompose cellulose, it is not unexpected to learn that fabrics may be weakened and destroyed by the action of microorganisms. Moist, warm conditions have been found to favor the action of fungi which are significant in this respect. One author has stated that the fungi first appear on the outside of the individual fibers and later send their mycelia through the canals of the cotton fibers. Mildew was found to be nearly proportional to the amount of food materials present in the fabrics. The growth of mildew was reduced by the removal of such food materials. Attempts have also been made to incorporate a disinfectant in the sizing. Such common bacteria as *Bacillus subtilis* and *Bacillus mesentericus* have been found to have no significance in the decomposition of textile fibers in fabrics.

BEVERAGES

Tea.—In the preparation of black tea a fermentation takes place which is mainly enzymatic. Bacteria are not desired and consequently every attempt is made to keep them out. The presence of bacteria means that there will be products of metabolism which may destroy the color.

Coffee.—The coffee bean contains much foreign matter besides the portion which is desired for the making of coffee. To remove the extran-

eous matter the fresh bean is subjected to a fermentation to loosen the material about the portion to be saved.

Cocoa.—For the preparation of cocoa, the seeds of the cocoa fruit are roasted and ground. About these seeds or beans is a great amount of pulp and extraneous matter which is removed most easily by fermentation. This fermentation is induced by piling the seeds on the floor where they heat and an active fermentation takes place. This fermentation also improves the flavor of the product. After the fermentation the beans are dried.

BREAD

The bread or panary fermentation is one of the earliest on which we have written records. It has been carried out in practically all countries. In general there are two kinds of bread, leavened bread and unleavened bread. Unleavened bread is made by mixing flour in water and then baking the resulting mixture. This gave, in olden times, a product not unlike the modern cracker except that coarser meals were often used. Later instead of baking the dough immediately after mixing, it was allowed to stand in a warm place until fermentation had taken place and the dough was full of gas. This gave leavened bread, the type now used in most countries.

Leavening Agents.—Two kinds are available, chemical leavening agents and biological leavening agents. Both cause the formation of gas in the dough or sponge. This gas makes a lighter and more delectable bread.

Chemical Leavening Agents.—The chemical leavening agents are those which cause gas formation when they are mixed into the sponge and the sponge is baked. These mixtures are generally called baking powders. One kind may be made by mixing two parts of cream of tartar (potassium acid tartrate) with one part of baking soda (sodium bicarbonate). Certain other substances may be added to prevent caking. The practice of using sour milk and baking soda also causes the formation of gas.

Vulfs and Vanderbilt in their Food Products enumerated the following advantages and disadvantages of chemical leavening agents:

Advantages:

1. The time is shortened. In a few minutes a light spongy dough can be prepared which would require hours by the use of yeast fermentation.
2. No loss of carbohydrate is involved.
3. It is possible to calculate the amount of gas which will be produced if the composition of the chemical agent is known.

Disadvantages:

1. The taste is not as good as that in products raised with yeast.
 2. The product is not as readily digested.
 3. The residue resulting from the chemical reaction remains in the loaf.
- As these residues have no nutritive value, they can only be regarded as waste products.

Biological Leavening Agents.—The biological leavening agents are microorganisms of different kinds. Yeast is probably the best known, although the bacteria have been found, in some cases, to function in a very desirable manner. In the use of yeast as a leavening agent, carbon dioxide is the desired product of fermentation, and not the alcohol as is the case in the fermentation for beverages. Pressed yeast is available in even the smallest hamlets in America. When the pressed yeast is not available dried yeast can usually be secured. In former times when distribution systems for pressed yeast were not so well developed as they are to-day, our forefathers had to make their own yeast.

The following advantages and disadvantages of biological leaven were enumerated by Vults and Vanderbilt:

Advantages:

1. Carbon dioxide is generated by the action of the yeast enzyme on the carbohydrate of the meal or flour and no foreign substance has to be added.
2. The slow liberation of gas causes it to have its full effect as a leavening agent.
3. The by-products produced during the fermentation give a pleasant taste.
4. Bread made with yeast is more digestible.

Disadvantages:

1. The time required for leavening is long.
2. Careful watching and study of those conditions necessary for the growth of yeast are necessary, else the fermentation will go amiss; sour bread may result.
3. A loss of carbohydrates takes place in the formation of the carbon dioxide.
4. As yeast is a living organism it is impossible to calculate the amount of carbon dioxide which should be produced.

When yeast is used as the leavening agent it acts on the carbohydrates contained in the dough. This sugar may be added to the sponge or it may be formed in the sponge by the action of the flour enzymes. Yeast itself does not contain amylase with which to decompose the starch to fermentable sugars. This explains how fermentation is accomplished in a sponge to which sugar is not added. After yeast has been added the

temperature is kept around 30° C. The little work that has been done on the effects of various yeasts indicates that the strain of yeast used does not influence the flavor of the bread.

Bacteria may also function as leavening agents. Good bread may be made with *Escherichia coli* as the fermenting agent. Koser found that *Clostridium welchi* was being used in a commercial starter for self-rising bread. This organism is an anaerobe and forms large amounts of carbon dioxide.

Bread is susceptible to several types of bad fermentations. One of the best known is *slimy* or *ropy* bread caused by the presence of aerobic spore-forming bacteria of the *subtilis-mesentericus* group. These organisms form spores which are so resistant to heat that they pass through the baking process and grow in the bread during storage. These organisms are capsulated and stick together giving the slimy appearance. *Moldy* bread is also quite common since bakeries may become contaminated with mold spores. Some molds grow on the surface of the loaf, giving it a chalky appearance. Sour bread is caused by the overgrowth of the yeast by undesirable bacteria. Red or bloody bread has been mentioned before as due to growth of bacteria which form red pigments; it is an uncommon abnormality in this country.

INDUSTRIAL ALCOHOL

Industrial alcohol was perhaps the first biological fermentation for the preparation of a chemical compound of industrial significance. It has had many uses in the past and has become very important. It may play an important rôle in the future as a fuel for the internal-combustion engine if the gasoline supply is exhausted. This would greatly increase interest in this substance.

Alcohol is made by a yeast fermentation of saccharine materials. These raw materials may be those which are naturally found in nature or they may be formed by the hydrolysis of more complex polysaccharides to fermentable sugars. The commonest raw materials have been potato starch and black strap molasses. The potatoes were thoroughly washed and cooked by high-pressure steam; after mashing, malt was added which converted the starch into fermentable sugars. Further manipulations involved fermentation of sugars with yeast with a subsequent distillation of the mixture to secure the alcohol.

When molasses is used, it is imported from sugar-producing countries, diluted with water and put into the fermenters. The yeast is added and the temperature controlled to make an active fermentation possible.

Most of the alcohol in America is made from molasses which is a waste product in the sugar industry.

In a more recent process molds are used for the hydrolysis of the starch instead of malt. This is known as the *amyl* process. The starch is cooked under high-pressure steam and then blown into the fermenters. These are large cylindrical tanks. The mold spores are added to the tanks and the temperature controlled until saccharification is accomplished. The yeasts used are those which have been used for years in the alcoholic fermentation; they have been trained to form large amounts of alcohol, at least much greater amounts of alcohol than are formed by the ordinary yeasts.

ACETONE AND BUTANOL

This is a more recent application of bacteria for the preparation of an industrially significant chemical compound. Before 1910 the literature of bacteriology contained several statements that bacteria could form acetone and butanol from starch. Fernbach, a French biochemist, isolated a microorganism which produced significant amounts of acetone. Since that time considerable study has been given the subject; to-day almost all of the acetone used in America is made by fermentation.

In former times most of the acetone was made by the destructive distillation of wood. In the biological preparation of acetone low-grade corn unfit for feeding purposes is the raw material. The corn is screened and ground. The corn meal is then mashed in tanks with a capacity of a little more than 10,000 gallons. After mashing, the corn is passed to the cookers, which are really autoclaves. Here it is heated for two hours with steam under pressure and finally completely sterilized by higher pressures just before the steam is shut off. The corn mash is then run into fermenters where it is inoculated with *Clostridium acetobutylicum* Weizmann. This organism changes the starch to normal butanol, acetone and alcohol. The bacterium converts 3 pounds of starch into 1 pound of mixed solvents. During this fermentation, gas composed of 45 per cent of hydrogen and 55 per cent of carbon dioxide is evolved. The solvent mixture is made up of 60 per cent of butanol, 30 per cent of acetone and 10 per cent of ethanol.² The mixtures resulting from the fermentation must, of course, be purified by chemical methods.

² Killefer, D. H. Butanol and Acetone from Corn. Ind. and Eng. Chem., **19** (1927), 46.

METHANOL

This product is made indirectly by fermentation methods. As stated above, in the discussion of the making of acetone and butanol a gas is evolved which contains hydrogen and carbon dioxide. As a method of utilizing these by-products, methanol is made by passing the mixed gases over heated carbon.

GLYCEROL

Small amounts of glycerol have been known for a long time to be formed in the fermentation of sugars. The exigencies of the World War made it necessary to seek other methods for the preparation of glycerol. A comprehensive discussion will not be attempted here. While a little glycerol has always been found in fermentations, it was discovered that the presence of a sulfite caused the course of the fermentation to be altered. Instead of the usual amounts of alcohol, glycerol was greatly increased.

The preparation of alcohol biologically was studied by several of the countries engaged in the War. Very soon after the Armistice, papers were published in scientific journals giving the details of the preparation. Two German investigators reported that when the weight of sulfite was twice that of sugar a yield of 36 per cent of glycerol was secured. Chapman, to whom this book is indebted for some of the facts presented here, stated that 1,000,000 kg. of glycerol were made by this fermentation each month in England. Eoff in America perfected a method by which 20 to 25 per cent of the sugar was changed into glycerol.

PRODUCTION OF FAT BY YEASTS

During the World War Germany became destitute of fat. Lindner, a German mycologist, isolated a strain of yeast which could convert other materials into fat. The organism grew on the surface of sugar solutions and produced growth of cells which contained 18 per cent of fat, 43 per cent of carbohydrates and 31 per cent of crude protein. This made it possible to convert materials of lesser value into material which, in the situation cited, was very valuable. Fats and proteins could be made in a quick and easy manner. Later investigations indicated that this yeast could be propagated in mineral salt-sugar solutions; from this fact it became known as "mineral yeast," having good characteristics for inclusion in the diet. This microorganism, then, in time of emergency would be a valuable one to possess. It has been shown by our Forest Products Laboratory at Madison, Wisconsin, that waste cellulose in

wood wastes could be hydrolyzed to fermentable sugars and since there are methods available for removing nitrogen from the atmosphere, the raw materials for the biological manufacture of fat by means of this so-called mineral yeast are always at hand.

LACTIC ACID

Lactic acid has great use in the industries. It has been prepared in different ways in the past. We need be concerned here only with the fermentation preparation, since the other methods of preparation will be found in chemical texts. Fermentable carbohydrates must be used as the raw materials. These are usually hydrolyzed starch, the hydrolysis being carried out either by acids and heat or by enzymes. The fermentation is carried out in the presence of calcium carbonate in order that the action of the bacteria will not be retarded by the accumulation of the acid. Different organisms are used, all belonging to the lactic acid group.

HYDROGEN

Another product of fermentation is hydrogen. As mentioned under Methanol, it is one of the by-products of the acetone-butanol fermentation industry. The hydrogen which is made in this way can be easily separated and has high purity. Thaysen reported that during the World War it was possible to produce 1000 cu. ft. (28.3 cu. m.) for twenty-five cents.

Carbon Dioxide.—This is coming to be an important by-product of the industrial alcohol industry. The carbon dioxide is collected from the fermenters and compressed in steel cylinders. Its best-known use is probably in carbonated beverages. It is also used for keeping certain cereals free from weevils. We have mentioned above the formation of large amounts of carbon dioxide in the acetone-butanol fermentation.

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*Asepsis =
absence of Bacteria*

CHAPTER XXIV

FOOD PRESERVATION

The increasing complexity of activities drove early man to food preservation. During very early times he was probably content with finding his food as he needed it, but as other activities began to encroach upon his time he found it more convenient to save the abundance of the hunt for a future need, and in this manner food preservation probably originated. Many foods are cooked to-day but if not cooked they are at least carefully washed. Food preservation industries are important adjuncts to present-day life since they make generally available foods which would ordinarily be attainable only at certain times of the year. They also allow the packing of matured fruit which, without the common methods of preservation, would have to be harvested green.

Asepsis. This is probably not an active method of food preservation but it may greatly affect the various procedures which may be used later. It has to do with the preparation of foods and the conditions of cleanliness under which they are handled. Modern hygiene and decency demand standards of cleanliness which our forefathers would have regarded as unnecessary. Aseptic preparation of foods is just as important for those which are to be preserved by one of the methods mentioned below as for those which are to be eaten fresh.¹ The cleaner the food and the freer from bacterial life, the lighter will be the load on the preservation method. We have already mentioned sanitary inspection in its relation to the production of clean milk and water. Similar attempts are made in the preparation and handling of most foods.

¹ The term "fresh" is often used in contradistinction to the term "preserved." Foods which are not "preserved" need not necessarily be fresh.



FIG. 122.—The Little Purple Stamp Used for Marking Carcasses and Products Passed by the Federal Meat Inspectors. (After Mohler, 1926.)

The three ciphers occupy the space where the number of the establishment appears.

A fine example of the effort to maintain asepsis in the preparation and preservation of foods is the inspection of meat by the federal government. This effort is visualized by the little purple stamp on meat. This stamp indicates that the animal from which the meat was prepared was examined before slaughter and that the meat was carefully



FIG. 123.—Federal Inspectors Making the Final Examination of a Beef Carcass.
(After Mohler, 1926.)

inspected before approval. If the inspectors, who are well-trained men, find an animal that looks suspicious, this animal is tagged with a tag which may read "U. S. Condemned" or "U. S. Suspect." Condemned animals must not be taken into the slaughter room; suspected animals are slaughtered separately and the meat is carefully examined before it is allowed to be prepared for sale. It should be stressed that not all

meat is inspected. The federal government has jurisdiction in interstate ~~matters only~~. Consequently only meat which is intended for interstate commerce can be inspected by the federal inspectors. It is unfortunate that all meat cannot be subjected to inspection. The opinion that home-killed meat does not need to be inspected may have arisen from malicious advertising of those who find it more profitable to buy animals locally for slaughter. Lack of space prevents a recital of the details of this attempt to conserve public health. The interested student will find them given in a very interesting manner by Mohler in a publication listed at the end of this chapter.

Low Temperature.—In Chapter X it was stated that low temperatures exert a marked inhibiting effect on bacterial life. This fact is made use of in the food industry; foods are kept at low temperatures in order to repress the development of the microorganisms contained in them. We learned that while cold kills some of the cells, a few always survive and grow very slowly. This means that foods in cold storage will not keep indefinitely. The low temperature may also have a bad influence on the types of bacteria that develop. In the case of milk experiments have shown that at low temperatures a proteolytic flora may develop instead of the normal carbohydrate fermenting and coagulating flora. The proteolytic bacteria digest the proteins in the milk and cause undesirable conditions to obtain.

Freezing.—This, of course, is the extreme application of cold. The water in frozen foods is crystallized and therefore not available for the bacteria. In this sense preservation by freezing is preservation by drying. No water is available for bacteria so long as foods are frozen. Meat may be preserved for a long time in the frozen condition. Fish and poultry are kept in this manner. The method has definite limitations and cannot be applied satisfactorily to fruits and vegetables.

Cold Storage.—This is probably the most important method by which great amounts of food are preserved by low temperatures. While it may be more expensive than some of the other procedures it does have certain advantages over some of them. Nothing is added to alter the appearance or condition of the food. Introduction of this method made available many foods which formerly could not be kept—cold storage has made apples available almost the entire year. It has also prevented great waste by saving the abundance of the harvest time for later periods when the fresh foods would be exhausted.

Different devices and procedures have been used for the preservation of foods by low temperatures, such as wet cloths, the cool cellar, the refrigerator, etc. The latter device is used in almost all American homes. The development of the so-called electric refrigerator may slowly revo-

lutionize refrigeration procedures in the home. These devices allow maximum refrigeration all of the time; this means safer foods and food in a better state of preservation.

Investigators at Columbia University have reported that the usual ice-cooled refrigerator was not an efficient apparatus. They suggested that each box be equipped with a thermometer for each shelf and that the upper limit of temperature be between 50° and 55° C. Such a procedure would allow the housewife to follow the temperatures which were being maintained in her refrigerator and indicate when they had risen to the danger point.

Period of Preservation.—How long may foods be preserved in the refrigerator? A general answer cannot be given to this question. A great many factors are involved. One case has been reported where a quarter of beef was kept in cold storage for fourteen years. When it was taken from the refrigerator and cut up for distribution the meat was found to be in good condition. Such periods of holding meat are, of course, unnecessary, but they do indicate the possibilities of proper methods of preservation. The fact that bacteria are known to develop slowly at temperatures just above freezing indicates that there are limitations.

High Temperatures.—The upper ranges of temperature are much more destructive to bacterial life than the lower ranges. In order to make use of high temperatures, various methods and devices are employed. Some of them, such as high-pressure steam, or modifications of them, have been described in the chapter on the Effect of Physical Agents on Bacteria.

Boiling and Cooking.—Boiling and cooking are methods of preparation and preservation. They are more commonly used as a preparatory step but function as important sanitary measures. While the application of heat to the food has probably, in many cases, prevented the dissemination of disease, the limitations should be understood. Different definitions may be used for the terms cooking and boiling. Cooking might vary from slight heating to prolonged boiling. Thorough cooking is one of the most satisfactory methods of rendering foods safer. Baking also has value in this respect. Information on the subject might be more apparent if a few experiments were quoted from the literature. One investigator studied the effect of broiling, roasting, and brazing on bacteria in and on meat. These methods gave unsatisfactory results because the heat did not penetrate. Another investigator confirmed these conclusions by using pieces of metal and tubes of chemicals the fusing points of which were known. These were inserted into the meat to determine the temperatures attained in different places. Another

worker boiled a leg of veal for three and one-half hours at 101°C ., and found that the temperature on the interior had reached only 89°C . A ham weighing 10 pounds after boiling at 102°C . showed a temperature of only 78°C . at the center. Another good illustration of the point that cooking does not always sterilize foods is given in Chapter XXVII where an account of a typhoid carried outbreak of typhoid fever is reported. In this case a viscous, pasty food preparation, spaghetti, resisted the penetration of that and allowed the bacteria to survive cooking.

The belief that certain foods are more nutritious or improved in flavor by insufficient cooking also has bearing on this discussion. Some believe that rare beef is more desirable than well-cooked beef. It is also believed that thorough cooking of oysters should be avoided else the oysters will be less digestible. In the latter case it is unfortunate that such ideas prevail for oysters are frequently harvested from polluted sources. They should be well cooked in order to make them safe at all times.

Cooking is especially significant in the destruction of toxins in foods. Such toxins have been important in America in connection with the several outbreaks of botulism caused by *Clostridium botulinum*. This toxin, like the toxins of all bacteria, is destroyed by heat. Boiling for only a few minutes detoxifies toxic material. One precaution, however, should be mentioned. Foods which contain toxin of this microorganism frequently contain large amounts of carbon dioxide. This is boiled off at temperatures considerably below 100°C . Consequently, such foods will have the appearance of boiling quite below 100°C .

Some experiments have been reported more recently which indicate that heat-resistant toxins may also be formed. These are spoken of as toxins but it might be better to use another term retaining the term toxin for the thermo-labile poisons.

Pasteurization.—This is the heating of foods to temperatures below the boiling-point but yet sufficiently high for the destruction of many of the bacteria. The idea of partially cooking foods by this process seems to have originated in the early days of bacteriology when it was realized that bacteria might be the cause of spoilage. These spoilages were called "diseases" of vinegar, wine, beer, etc. Heating of these substances to 60°C . was found to destroy the organisms which were involved in the spoilage and to make them keep longer. This is especially true for the fruit juices such as grape juice and apple juice. The best-known case of the use of pasteurization is in the milk industry where it is used to destroy among others the microorganism causing tuberculosis. It should be pointed out that pasteurization does not

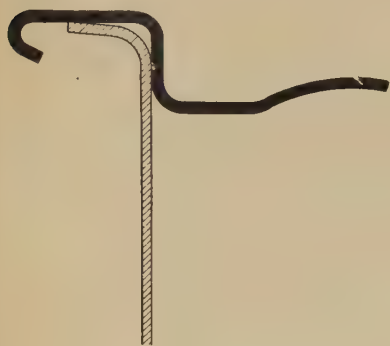
sterilize and that many bacteria may be present; but, if the process is carried out in such a manner that the material is brought to 60° C. and held at that temperature for the required period, there will be a great reduction in the numbers of bacteria.

Canning.—The sterilization of foods in sealed containers is one of the commonest methods of food preservation. Nicolas Appert is given credit for the discovery of canning as it is known to-day. In 1810 he received the prize of 12,000 francs which the Minister of the Interior, Montalivet, had offered for a method of food preservation. This prize was the result of concern which Napoleon I felt over the provisioning of the French fleet. Scurvy and other diseases of malnutrition had afflicted the sailors. In 1750 Appert, when 60 years old, built a factory of his own; this suffered from the wars which followed the above date. It was entirely wiped out later. Canning has made available to the consumer all sorts of foods out of season. There is scarcely a food which cannot be secured to-day in tin.

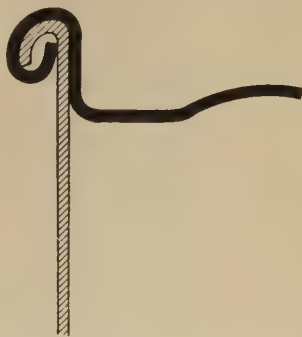
The term "canned" has several meanings. Some use it for all methods by which foods are packed in sealed containers irrespective of the type of container used. Others have suggested that the term canned be applied only to foods packed in tin. In this discussion no attempt will be made to distinguish these conceptions sharply.

The containers may be made of either glass or tin; tin containers are more commonly used under commercial conditions while glass containers are used under domestic conditions. Both types may be sterilized in the same manner under pressure if desired although the glass container gives more breakage. Two types of tin cans have been used. One is known as the "hole-and-cap" can and the other as the "open-top" can. The latter type is also known as the sanitary can. The former has a hole in the top of the can over which fits a solder-hemmed cap. The solder on this cap is fused on the closing machine and the can sealed in this manner. This type of container is not used as much to-day as formerly on account of the greater advantages inherent in the open-top can. The cover is fastened to the open-top can by a "double seam" shown in Fig. 124. Between the layers of metal is a paper gasket which helps to make the seam tight. No solder is used in closing this can as is the case with the hole-and-cap can. Tin cans are made from steel rolled very thin and covered with a coating of tin. Despite the general belief that the coating of tin is important, experiments have indicated that it is relatively unimportant. Some believe that illness is more liable to result from eating foods from cans with too little tin. There is no foundation for this belief. Some cans are lacquered or enameled on the inside and are spoken of as inside-enameled cans. It is

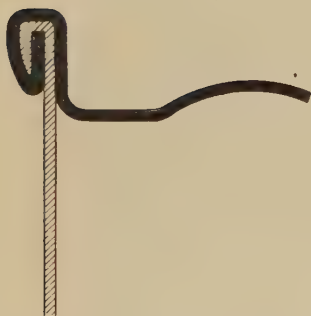
stated by some authors that these cans are used to prevent corrosion or pin-holing. With one or two exceptions this is not the reason for the use of these cans. They are used to preserve the natural color of the foods packed therein. Such foods as cherries, beets, etc., are packed in



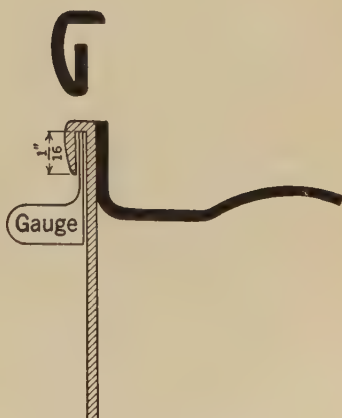
Position of Cover and Flange
Before Seaming.



Correct Curling or First Operation
Seam.



Correct Second Operation Seam.



Showing Proper Method
of Measuring Hook.
Use Flexible Steel Scale.

FIG. 124.—Showing the Manner in Which the Cover is Applied to an Open-top Type of Can.

such cans. With some foods lacquered cans perforate more rapidly than plain cans since there may be spots where the lacquer has not completely covered the tin. The contents of the can will then exert an intensive action on such places.

Procedures in Canning.—*Raw Materials:* These should be the best both with regard to quality and state of maturity. Canning factories should be located as near the production fields as possible. This makes it possible to use fresh foods which have not had opportunity to spoil or even become old. In the fish-canning localities, canneries have been constructed on boats which may be moved from place to place and kept close to the source of supply.

Preparation.—This depends entirely on the product. Fruits are generally quite clean and are treated to remove foreign matter. Fruits with skins are washed and sometimes peeled. Tomatoes from clay soils are washed more carefully than those from sandy soil. Some vegetables require hand washing.

Grading.—This is employed in order to give a uniform product. In some cases it is greatly overdone. There are about 12 grades of peas, an equal number for cherries and 18 for peaches. Grading is done mechanically.

Blanching.—This is a brief exposure to hot water or steam just before the food is placed in the can. It is used for removing sticky matter on the outside of the vegetables or fruits as well as to reduce the volume. The term might suggest that it is employed to whiten the food product. Such is not the case.

Filling the Can.—With such materials as corn and peas, filling is done mechanically with machinery. Other foods, however, such as pickles, meats, etc., may require hand filling. Hand filling is necessary where the foods have to be layered into the can. After the solid portion has been placed in the can, a syrup or brine may be added.

Exhausting.—This involves heating the filled can to drive out the air and raise the temperature as high as possible before closing. This is one of the most important steps in canning; some of the defects of canned foods may be traced to improper exhaust. For instance, oxygen has been found to accelerate perforation and to reduce the vitamins. These defects are greatly reduced, if not entirely avoided, by a satisfactory exhaust.

Processing.—Processing involves the application of heat to the container into which the food to be preserved, has been placed and sealed. Under some conditions, especially those which may obtain in the home, the container is only partially sealed, final sealing being done when the container is removed from the sterilizer. The term "processing" should be distinguished from the term "sterilization." They are probably not synonymous. A processed food is not necessarily a sterilized food. Canned foods are not always sterile. In fact two investigations made on the sterility of canned foods purchased on the open market revealed a

rather high percentage of unsterile cans. The viable bacteria which were found to be present were probably in the spore stage and were dormant in the cans. They would be of no significance unless they were spores of some of the organisms forming toxins. This is not probable.

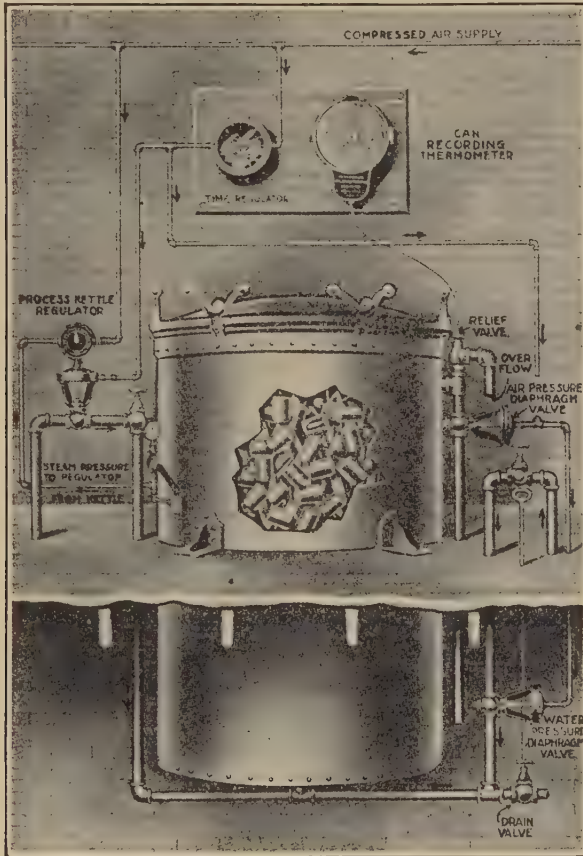


FIG. 125.—Showing a Typical Retort or Cooker with Regulators for Controlling the Temperature, Fixing the Time and Cooling the Product when the Process is Completed. (After Bitting.)

The results of the process depend largely on the condition of the raw materials which were canned, the penetration of heat into the can and other factors. When the raw materials are fresh and clean, fewer bacteria get into the can and the process is more efficient. Incipient spoilage means a rich growth of bacteria which have to be killed. We have learned that the rate of disinfection depends on the number of

living cells. When the number is low, disinfection is more easily and quickly accomplished than when the number is great. The same situation exists in the processing of foods.

Cooling.—Cooling is applied to stop the action of the heat. If the cans are not cooled immediately after leaving the retort they remain hot for long periods and the cook may continue for some time. Cans are cooled by drawing them through water on a belt or by piling on a platform with a slat bottom. Insufficient cooling often results in the spoilage of the food product later by thermophilic bacteria which may survive the process.

Period of Preservation.—How Long Will Canned Foods Keep? Since canned foods are supposed to be sterile or at least contain bacteria in a dormant condition, they should keep as long as these bacteria do not grow. With some foods chemical reactions may take place between the contents and the metal of the container. These types of spoilage will not be discussed here. A number of instances are on record where canned foods have been opened a great number of years after packing and were found to be in satisfactory condition. One of these involved a case of peas which had been mislaid for twenty-four years. Another case recently reported in the literature concerned a can of beef which was included in the stores of an exploratory voyage of Sir John Franklin. The expedition left England in 1845 and the whole crew perished in the Arctic regions. Rescue parties located an abandoned sledge on which this can of beef was found. The can remained in Liverpool from 1888 until 1926. It was then opened in the presence of a bacteriologist and other food experts. The contents was found to be in apparently perfect condition. Laboratory examination of the contents revealed no living bacteria and there was no evidence of illness among those who partook of the meat. Other instances could be mentioned but these two should suffice. These cases do not indicate that old canned foods are desirable. It would be a fine thing if the date of packing could be placed on all cans of food. This would be in keeping with the spirit if not the letter of the various food and drug laws. Furthermore, it should not affect the sale of canned foods.

The canning industry has reached a very high state of development in America. There is scarcely a food that may not be secured packed in tin containers. Canning has made possible the use of certain foods the year round as well as the use of tropical fruits which otherwise might not be available.

Home Canning.—Domestic methods of canning are not greatly different from those used in the large canning factories. In the home, however, grading, washing and sorting are performed by hand. Process-

ing may be quite different. Two general methods have been used, high-pressure steam and boiling water. The first method is applied in the home by means of pressure cookers, contrivances for accomplishing in the home what is accomplished in retorts under commercial conditions. The temperatures used depend on the food product and vary between 100°C . (212°F .) and 121.5°C . (250°F .). Processing in boiling water has been somewhat replaced by the pressure cooker; the latter method gives more reliable results. When boiling water is used, the containers

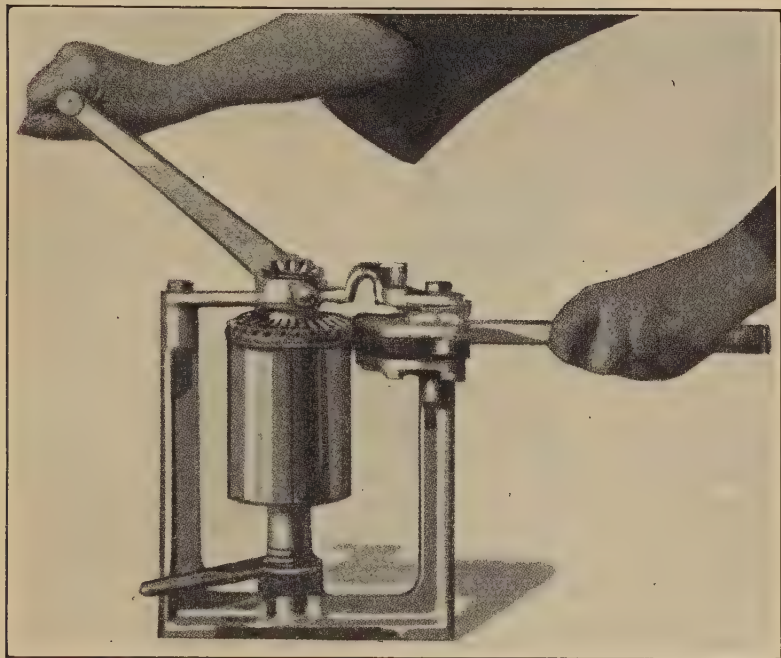


FIG. 126.—Sealing Sanitary or Open-top Cans with a Hand Sealer. (After Stanley.)

are placed in the water for lengths of time which have been found to be adequate. The method may be continuous or intermittent. Experts of the United States Department of Agriculture have published the following time-tables for processing foods under domestic conditions in boiling water and under pressure in the cooker.

The containers used by the housewife are usually made of glass. Tin cans, however, have come into use since hand sealers have been devised. Details of home canning procedures may be found in numerous publications of both the federal bureaus at Washington as well as those of the various Agricultural Experiment Stations.

TIME-TABLE FOR CANNING FRUITS, TOMATOES, PICKLED BEETS, AND PIMIENTOS

The times given for processing in boiling water apply only to places with altitudes of 1000 feet or less. For all altitudes above 1000 feet the time should be increased 20 per cent for each additional 1000 feet.

When half-gallon glass jars are used, add five minutes to times given for pint and quart glass jars.

Product	Method of Treatment before Processing	PROCESSING PERIOD IN BOILING WATER	
		Pint and Quart Glass Jars. Period in Minutes	No. 2 and No. 3 Tin Cans. Period in Minutes
Apples.....	Slice, quarter, or halve, then pack in containers and cover with boiling sirup.	15	10
	Or boil whole in sirup, or bake as for serving, and cover with sirup, and pack hot.	5	5
	Or pack hot in form of apple sauce	5	5
	Same as peaches.		
Apricots.....	Pack in containers. Fill with boiling hot, medium sirup. Or precook and pack hot.	20	15
Blackberries.....		5	5
Blueberries.....	Pack in containers, cover with boiling sirup, using thick sirup for sour cherries, and medium for sweet.	25	20
Dewberries.....		5	5
Huckleberries.....	Or remove pits, add sugar as desired, bring to boil, and pack.	5	5
Logan blackberries.....		5	5
Raspberries.....	Same as berries.	5	5
Cherries.....	Sprinkle 1 cup of soda over 6 quarts of figs. Add 1 gallon of boiling water. Allow figs to stand in this five minutes. Drain and rinse well. Add 2 quarts boiling medium sirup. Boil for one hour. Fill in containers. Cover with hot sirup.	5	5
Currants.....	Pack in containers. Fill with boiling hot, thick sirup.	20	15
Figs.....	Or prepare sauce, using sugar as desired. Fill hot.	5	5
Gooseberries.....	Scald, dip into cold water, and peel. Cut into size desired, removing pits. Fill containers, then add sirup of desired consistency, in which one cracked peach pit for every quart of sirup has been boiled.	20 for ripe fruit, 25 for firm fruit.	15
Peaches.....	Pare and cook for four to eight minutes in boiling medium sirup. Pack hot in containers and fill with the boiling sirup.	20	20
Pears.....	Peel, core, remove eyes. Cut into convenient cross-sections. Pack in containers. Fill with boiling thin sirup.	30	25
Pineapples.....	Prick. Fill in containers. Cover with boiling medium sirup.	20	15
Plums.....	Or bring to boil, using sugar as desired. Fill hot into containers.	5	5
Rhubarb.....	Cut in half-inch lengths. Add one-fourth as much sugar as rhubarb by measure. Bake until tender in covered baking dish. Pack in hot containers.	5	5
	Or pack uncooked with boiling sirup.	20	15
Strawberries.....	To each quart add 1 cup of sugar and 2 tablespoons of water. Boil slowly for fifteen minutes. Let stand overnight in the kettle. Reheat to boiling. Fill containers hot.	5	5
Tomatoes.....	Scald and peel. Pack, whole or cut in pieces. Cover with hot tomato juice. Add 1 teaspoon salt to each quart.	45	35
Pickled beets.....	Precook, peel, and slice in containers. Cover with mixture of vinegar and sugar, boiling hot.	30	30
Pimientos.....	Heat in hot fat or oven to loosen peel. Peel and pack in small containers. Add one half teaspoon salt to each pint.	40 for pint glass jars.	30 for No. 1 or No. 0 tins.

TIME-TABLE FOR CANNING NON-ACID VEGETABLES WITH THE PRESSURE CANNER

Pack vegetables as nearly boiling hot as possible, using additional boiling water if necessary. Add 1 teaspoon salt to quart to all vegetables, and 2 teaspoons sugar, if desired, to corn. Place jars or cans in hot canner as soon as they are filled.

Product	Method of Treatment Before Processing	PROCESSING PERIOD IN PRESSURE CANNER		
		Quart Glass Jars	Pint Glass Jars	No. 2 and No. 3 Tin Cans
Asparagus...	Tie in uniform bundles, place in saucepan with boiling water over lower t o u g h portion, cover tightly, boil four to five minutes, and pack hot into containers. Or cut in half-inch lengths, bring to boil in water to cover, and pack hot into containers.	40 minutes at 10 pounds pressure, or 240° F.	35 minutes at 10 pounds pressure, or 240° F.	30 minutes at 10 pounds pressure, or 240° F.
Beans, string..	Heat to boiling with water to cover. Pack hot into containers.	40 minutes at 10 pounds pressure, or 240° F.	35 minutes at 10 pounds pressure, or 240° F.	30 minutes at 10 pounds pressure, or 240° F.
Beans, lima...	Can only young and tender beans, using method suggested for peas.	60 minutes at 10 pounds pressure, or 240° F.	55 minutes at 10 pounds pressure, or 240° F.	50 minutes at 10 pounds pressure, or 240° F.
Baby beets...	Can only young tender beets. Scald in boiling water or steam until the skins slip easily. Skin and pack hot into containers.	40 minutes at 10 pounds pressure, or 240° F.	35 minutes at 10 pounds pressure, or 240° F.	30 minutes at 10 pounds pressure, or 240° F.
Corn.....	Cut off without precooking. Add half as much boiling water as corn by weight, heat to boiling, and pack hot into containers.	80 minutes at 15 pounds pressure, or 250° F.	75 minutes at 15 pounds pressure, or 250° F.	70 minutes at 15 pounds pressure, or 250° F.
Greens, including spinach..	Steam or heat in covered vessel until completely wilted, using just enough water to prevent burning. Pack hot into containers, taking care that the material is not packed too solidly and that there is liquid to cover.	90 minutes at 10 pounds pressure, or 240° F.	85 minutes at 10 pounds pressure, or 240° F.	80 minutes at 10 pounds pressure, or 240° F.*
Okra.....	Can only young, tender pods. Cover with water and bring to boil. Pack hot into containers.	40 minutes at 10 pounds pressure, or 240° F.	35 minutes at 10 pounds pressure, or 240° F.	30 minutes at 10 pounds pressure, or 240° F.
Peas, green...	Use only tender young peas. Bring to boil with water to cover and pack hot into containers.	50 minutes at 10 pounds pressure, or 240° F.	40 minutes at 10 pounds pressure, or 240° F.	30 minutes at 10 pounds pressure, or 240° F.
P e a s , black-eyed	Same as lima beans.			
Sweet potatoes	Boil or steam until skins slip off readily. Peel quickly and pack hot into containers.....	60 or 70 minutes at 10 pounds pressure, or 240°	50 minutes at 10 pounds pressure, or 240° F.	No. 2 cans 50 minutes and No. 3 cans 60 to 70 minutes, at 10 pounds pressure, or 240° F.

*Should not be canned in No. 3 cans because of difficulty of heat penetration.

Criteria of Satisfactory Canned Foods.—Certain precautions if properly observed will make canned foods perfectly safe. A sound can of food will have concave ends. This does not mean that every can of food with bulged ends is dangerous and will cause illness if eaten. Such cans are abnormal and should be discarded. Ignorance of this or carelessness has caused some of our worst outbreaks of food poisoning. Some cans of spoiled food may not show evidences of spoilage since the bacteria concerned might not form gas. In many cases such cans will possess other evidences of decomposition such as bad odors or abnormal appearance. Such cans of food may be just as dangerous as those which have bulged ends. It is a significant fact that almost all of the outbreaks of botulism have been caused by foods which were recognized to be abnormal in some respect. Those who were concerned stated that the foods "bit the tongue," "smelled bad," or had a bitter taste. Consequently, outbreaks of botulism have been due to the carelessness of those who were affected. Thorough boiling would render all canned foods safe. Thom and Hunter of the Bureau of Chemistry have advised that the contents of all cans of food be boiled before use irrespective of whether there were signs of abnormality or not. The United States Department of Agriculture has discussed this question as follows:

It is the opinion of most bacteriologists that we have not much occasion to dread food poisoning from canned food provided we make it a rule to look at and smell carefully every can of food when it is first opened. The canning factory maintains a rigid inspection; why not introduce an equally good inspection service into your own home. Begin by inspecting the container before it is opened. If it is a glass jar look for gas bubbles and note whether the product seems to have changed color or become mushy. The lid should require the application of some force to remove it, for the sealing of the jar while its contents are boiling hot results in the formation of a partial vacuum, and if the seal remains perfect the pressure of the surrounding atmosphere holds the lid down. Similarly the tin can should be flat or slightly drawn in at the ends when cool. If swelled or bulged the probabilities are that the contents have spoiled as a result of the action of gas-forming organisms. It is a safe rule to discard, without tasting, any canned food which has conspicuously softened or has become mushy to an extent not warranted by the cooking process to which it was subjected; or which contains gas bubbles; or which has a peculiar or unusual smell. If the canned food successfully passes all of these tests but is found to possess an unduly sour taste, or an unusual flavor of any kind, it should at once be rejected; and the portion tasted should also be rejected without swallowing, to prevent any possible danger of poisoning.

It is a fortunate fact that spores are not as a rule found in those disease-bearing bacteria which are at all likely to occur in canned products, so far

as we know at present. The exceptions to this statement are so rare that they should not properly be used as an argument against either commercial or home canning. One hears a great deal about *Bacillus botulinus* poisoning from canned and bottled goods; the danger is a real one in some localities, yet the actual number of outbreaks of botulism at the present date is exceedingly small in comparison with the number of people who consume canned goods. Furthermore, botulinus experts among the bacteriologists state that botulism from canned products can be guarded against by use of four simple expedients, which are:

(1) Make it the absolutely invariable rule never to can any vegetable or fruit not in first-class condition; that is, do not can food which is slightly moldy or specked, oversoft, or "just ready to spoil," or partly rotted. Cutting out the soft spots and using the rest for canning may prove very poor economy in the end.

(2) Give all canned food a careful and rigid inspection *at the time the can or jar is opened*, and discard any material having an unusual appearance or odor, *without even tasting of it*. It is a useful precaution to notice the odor of the vegetable while it is boiling; for an odor so slight as to be unnoted while the vegetable mass is cold may be quite plainly perceived when intensified by heating.

(3) *Boil the food as it comes from the can before tasting it*. In this type of poisoning, fortunately, most bacteriologists believe that it is not necessary to destroy every bacterial spore, but rather the toxin or poison formed by the growth of the bacteria, which is a much easier matter. It must be clearly understood, however, that this does not warrant us in boiling spoiled food and then consuming it.

(4) The final disposition of canned foods which have spoiled or are suspected of spoilage is a matter of real importance. Chickens and other animals may be and often have been fatally poisoned by eating such spoiled materials. Even worse than this danger is the possibility of spreading *Bacillus botulinus* (or possibly other dangerous spores) through soil. With such considerations in mind it would seem that spoiled canned goods should be treated as we should treat any sort of infectious material, such as discharges from a typhoid or tubercular patient or the carcass of an animal which has died of anthrax; that is to say, they should be burned, or, if that is impracticable, they should be boiled for an hour with some efficient disinfectant in order to be sure that all dangerous spores are destroyed. Burying them deeply in soil with a generous covering of quick lime will prevent the poisoning of domestic animals and may have some influence in preventing infection of the soil with a highly dangerous organism.

Spoilage of canned foods may result from different causes. Unfit raw material may have been used or faulty methods employed. These may be faulty in some step before the food enters the can or the cans may not be properly closed. In the latter case bacteria may be drawn into the can at some time during storage; those who examine cans of food

bacteriologically must decide whether the bacteria were in the can when it was closed or whether they gained entrance afterward. This is often a difficult thing to do. Different terms are used for describing the several types of spoilage. Swells are cans in which generation of gas has taken place to such an extent that the ends are bulged. The gas may be due to bacterial activity or to chemical reaction between the contents of the can and the metal. In the latter case the gas is usually hydrogen. A flat sour is a can in which the contents has soured due to the activity of so-called "flat sour organisms." There are usually no external evidences of spoilage. The ends of such cans are flat but the contents possess a sour taste due to acid from bacterial growth. Other terms have been introduced to describe modifications of these conditions. They are not especially useful and need not be mentioned here.

Canning Powders.—These have been advertised as aids in the canning of foods especially under domestic conditions. The Bureau of Chemistry studied one such powder and found it to be composed of 5 per cent of table salt and 95 per cent of boric acid. This powder was found to have little influence on the keeping qualities of canned foods; an excess of the powder was said liable to be harmful, and lead to the slighting of other steps known to be necessary in the packing of good foods. Such powders are unnecessary in canning.² If the raw materials are of good quality and if they are properly washed and prepared for the can, and if they are properly processed and cooled in accordance with well-established practices, there should be no difficulty in obtaining cans of food that will keep and be satisfactory in every respect. In the case of the canning powder mentioned above, it contained a chemical boric acid, which has been prohibited by the government as a food preservative. It has been more recently prohibited in England, also.

Preservation of Food by the Addition of Chemicals.—Some chemicals are harmful to bacteria; this caused their introduction to foods as preservation agents. The great difficulty, however, is to secure a chemical compound which is harmful to bacterial cells and not harmful to those who partake of the foods. This difficulty is illustrated by the fact that many compounds have been tried for a while only to be prohibited later. Some of these compounds are sulfur dioxide, salicylic acid, boric acid, etc. The Food and Drug Act of 1906 states that any food which contains "added poisonous or other deleterious ingredient which may render such article injurious to health" is adulterated. The interpretation of this part of the law has caused considerable controversy and soon after the food and drug act was passed experiments had to be carried out to test the poisonous properties of several chemical compounds.

² Thom and Edmonson. Circular 237, U. S. Dept. of Agriculture.

Sodium Benzoate.—Much controversy and investigation have centered about the use of this compound as a food preservative. Dr. Wiley, when chief of the Bureau of Chemistry and since then, has argued that this compound should be prohibited since it was a poison and since, if the foods were in a good condition when preserved, such a preservative would not be necessary. On account of great difference of opinion, President Roosevelt appointed a referee board to carry out experiments testing the poisonous properties of sodium benzoate. Folin, to whom this book is indebted for some of the material presented here, gave the conclusions of the first report of the referee board as follows:

1. Sodium benzoate in small doses (under five-tenths of a gram per day) mixed with the food is without deleterious or poisonous action and is not injurious to health.

2. Sodium benzoate in large doses (up to four grams per day) mixed with the food has not been found to exert any deleterious effect on the general health, not to act as a poison in the general acceptance of the term. In some directions there were slight modifications in certain physiological processes, the exact significance of which modifications is not known.

3. The admixture of sodium benzoate with food in small or large doses has not been found to affect injuriously or impair the quality or nutritive value of such food.

Dr. Wiley and his colleagues disagreed with these conclusions. They stated that the addition of sodium benzoate was highly objectionable since it disturbed metabolic functions and caused injury to health. It is difficult to reconcile the conclusions reached by the referee board with those reached by Dr. Wiley. A German investigation on the poisonous properties of sodium benzoate as a food preservative found that when sodium benzoate was administered in sufficiently large amounts, it caused illness in dogs. It required four-tenths of a gram of benzoic per pound of dog to cause symptoms of illness. Folin stated that among all of the preservatives of recent origin there is not one more likely to prove practically harmless to human beings than benzoic acid or benzoates. He called attention to the fact that certain food materials, cranberries for instance, contain benzoic acid or substances which give rise to it in the human body.

The benzoates at best are feeble preservatives. Sodium benzoate is a permitted preservative in America. Studies with pure cultures of microorganisms, yeasts and bacteria, in fresh apple juice, and the like, showed practically no preservative action.

Boric Acid.—As stated before in the chapter on the Action of Chemical Agents on Bacteria, boric acid is a very feeble antiseptic. Boric acid and its compounds are not permitted in America as food preserva-

tives on account of their poisonous properties. In the literature on food poisoning there are frequent references to outbreaks of illness which have been traced to boric acid in foods. All of the nations have barred it from the list of permitted food preservatives. In the case of Great Britain boric acid was not put on the prohibited list until after a thorough investigation.

Salting and Pickling.—Common table salt, sodium chloride, has been extensively used as a food preservative.

Common salt is the name given to the native and industrial forms of sodium chloride. This substance is widely distributed in nature, occurring in deposits and also in sea water. Although widely distributed in nature, salt appears to have been quite unattainable to primitive man in many parts of the world. Thus the Odyssey speaks of inlanders who do not know the sea and use no salt in their food. In some parts of America and India, salt was first introduced by Europeans; and there are still parts of Central Africa where its use is a luxury confined to the rich.

Since salt appears to have become a necessity very early in the life of nations, it has played an important part in commerce and religion. Some ancient tribes regarded a salt spring as a gift of the gods. The Germans once waged war for saline streams and believed that the presence of salt in the soil invested a district with a peculiar sanctity and made it a place where prayers were most readily heard. The gods were worshipped as givers of kindly fruit, and salt and bread was a common phrase all over the world, being habitually associated with offerings. Homer calls salt divine and Plato names it a substance dear to the gods. Covenants were frequently made with salt as a necessary element; its preservative qualities made it a fitting symbol of enduring compact and fidelity. Every meal which included salt had a sacred character and created a bond of piety and friendship between the guests. Hence the expression "there is salt between us," and the phrases "to eat the salt of the palace" and "untrue to salt." Early in the history of the Roman army and in later times—salt was rationed out to the soldiers.

The beginning of commerce was perhaps a traffic in salt. One of the oldest roads in Italy was built primarily to carry salt into the interior of the country. In Phœnician commerce, salt and salt fish always formed an important item. The economic importance of salt was further indicated by the salt taxes. This often resulted in the salt reaching the consumer in a very impure state; hence the expression in Matthew "the salt which has lost its savour." This may be regarded as a very early attempt at food adulteration. Cakes of salt have been used as money in certain parts of the world. Even down to the present time salt has been used as a medium of exchange among various tribes. The Roman soldier was given a "Salarium," which was a money allowance to purchase salt. This is probably the basis of our modern term "salary."

Sauerkraut.—This food is prepared by fermenting cabbage tissue under conditions which allow the formation of organic acids; these

prevent putrefaction in the same manner as they do in other cases already mentioned. In this respect sauerkraut has much in common with cucumber pickles and silage. In the preparation of sauerkraut cabbage is shredded and then tightly packed into barrels or tanks with alternate layers of salt. From 3 to 5 pounds of salt per pound of cabbage are used. This salt draws the water from the cabbage tissue to make a brine. In this brine lactic acid bacteria grow and ferment the sugars which have diffused from the cabbage. The acids formed are probably lactic and acetic mainly. One difficulty in making sauerkraut is the presence of objectionable microorganisms, such as *Oidium lactis*, which eat the acid and make it possible for the putrefactive bacteria to develop. Abnormal fermentations are not uncommon and give a kraut with bad flavor. Pure culture inoculation with desirable bacteria has helped to raise the quality of sauerkraut.

Cucumber Pickles.—Another vegetable preserved by salting is the cucumber. Special species are grown for preserving and the manufacture of pickles. When harvested they are taken to salting stations where they are dumped into large tanks of salt and water brine. This brine to start with is about 20 per cent concentration. In such a concentration the cucumbers float and have to be "keyed down" by putting a slat cover over them so that all of the cucumbers are kept under the brine. If any remain out of the brine or partly out of it, they undergo spoilage and have to be discarded. The strong brine draws the sugar out of the cucumber which slowly changes into a pickle. The difference between a cucumber and a pickle is easily discernible by comparison. During the first few days the tank appears to boil, so active is the fermentation. After this initial fermentation the action slows up and the acids, formed during the fermentation, function as agents of preservation.

The cucumber undergoes distinctly visible changes during the fermentation process. These changes have assumed some importance to the pickle maker who uses them as criteria for judging the progress of the curing process. These changes may be summarized as follows:

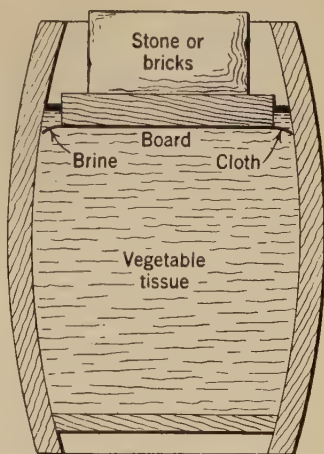


FIG. 127.—Showing the Method of Packing Vegetables into the Keg for Fermenting or Salting.
(After Round and Lang.)

1. A change in color from the bright green to a duller olive green.
2. A change in the cross-section of the cucumber which is first a snow-white to a more waxy-partially-transparent color or appearance.
3. A change in the specific gravity; the cucumbers first float and have to be keyed down to keep them under the surface. Later as the during has progressed they become heavier than the brine and remain submerged.

The change in the color from a bright green to a duller green is probably due to the action of the acid on the chlorophyll. This can be shown by soaking cucumbers in acetic or lactic acid. In a short time they assume the appearance of a cured pickle while controls require the usual longer time if they are allowed to cure by fermentation.

The second change is largely due to a replacement of the air in the cucumber by other liquids. Fresh cucumbers always contain a large amount of air which can be easily demonstrated by putting them under a high vacuum. The air imparts to the cucumber a snow-white opaque appearance which changes to a waxy, partially-transparent appearance after curing.

The change in the specific gravity depends on the diffusion of salt into the cucumber and also on the progress of the fermentation. During the first few days of their fermentation there is probably an active osmosis by which the character of the cucumber is markedly altered. After the cucumbers have been properly fermented they may be kept for a long time.

Salting of Meats.—Meats have been preserved for a long time by means of salt-sugar solutions. The meats may be placed for a considerable time in these solutions and later subjected to other procedures such as smoking. Larger pieces of meat may be preserved by rubbing the salt in by hand.

Salt exerts a repressing action on bacterial life best explained by plasmolysis, a phenomenon which has already been discussed. The taste of the salt in foods preserved with it is often objectionable.

Spices.—Although these condiments are added mainly to improve the flavor of the food, some of them have distinct antiseptic value. Cinnamon and cloves are germicidal while pepper and ginger have been found to be practically devoid of action on bacteria. The keeping qualities of a number of foods are greatly enhanced by the presence of spices which are primarily added for flavor. This is probably the situation with ketchup, mincemeat and such foods.

Smoking.—Preservation of meats by smoking is really a method involving the use of chemicals. In this case the chemicals are sub-

lined on the meat from smoke, whereas in the other procedures they are added directly. The compounds which preserve meats in smoking are creosotes made by causing wood and other materials to smolder in a confined space. In addition to their preservative effect, these compounds also alter the flavor.

Chemicals Formed in Fermentation.—In this paragraph we will summarize some of the information scattered through several paragraphs above. In the case of fermentation the chemicals used as preservatives are made by the bacteria themselves. The desirable bacteria have to be favored in some way and the extraneous forms kept out. Vegetables are preserved by salting to accomplish these results. The salt represses those bacteria which cannot tolerate it but allows the lactic acid forms to develop. Bacteria which can tolerate fairly high concentrations of salt are called halophilic bacteria.

Drying or Dehydration.—It has been stated that water is quite necessary for bacterial growth and metabolism. Bacteria are not completely destroyed by the absence of water but there is probably a great reduction in numbers and activity. This fact explains how foods may be preserved by drying and dehydration. This method was used by our forefathers for the preservation of several types of foods. Nature uses this method for preserving starch in the grains. A great variety of food is preserved in this manner to-day. During the World War a wider variety was preserved in this manner. Most attention has been given to the fruits. During the World War dried vegetables were prepared and shipped to our army. Drying has several advantages over other methods of food preservation. The weight and bulk are markedly reduced—an important factor in the shipment and storage. Certain disadvantages must also be admitted. The flavor is altered to some extent and such foods must be soaked and cooked before eating.

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Idiosyncrasies

CHAPTER XXV

ILLNESS CAUSED BY FOODS (FOOD POISONING AND FOOD INFECTION)

This is one of the most striking examples of the possible harmfulness of bacteria. They may grow in foods and excrete products which are harmful or form harmful compounds from the foods themselves. All degrees of illness are included under the diagnosis of food poisoning. Some of the information on food poisoning rests on well-established facts; some, however, does not. Some food poisoning outbreaks have been satisfactorily studied while the conclusions reached in other outbreaks seem ill-founded. Diagnoses of food poisoning are sometimes made when other reasons are not apparent to which the illness could be ascribed.

Care of Food Products.—While great advances have been made in the education of food handlers, one does not have to be very observing to see quite objectionable practices. Personal cleanliness is more necessary on the part of those engaged in preparation of food than in any other occupation. In the average home this matter is probably given proper attention; in public dining-rooms, however, even in pretentious hotels, dining-cars, etc., one may notice questionable actions on the part of those who serve. Finger bowls are used repeatedly without adequate washing and sterilization. Soiled napkins and towels may be used for wiping tableware. While our state laws govern the production and transportation of foodstuffs, relatively little attention is given the conditions under which such food is prepared for the public. Several years ago, a rather large outbreak of food infection was traced to an uncooked salad prepared by a female employee of a dining-hall whose duty it was to mop the floor and aid in the dispensation of food. Thus it is seen that the channels of infection are frequently very direct between sources of pollution and the food product.

ALLERGIC REACTIONS — IDIOSYNCRASIES

These are constitutional peculiarities which some people exhibit towards certain foods. They are often spoken of as allergic reactions of

foods, another example of anaphylaxis. These food idiosyncrasies are often quite serious and may be diagnosed as other ailments. Or, they may be slight or scarcely perceptible. A wide variety of foods may cause these reactions. Allergic actions are usually explained on the basis of proteins which are contained in the foods.

They are due to anaphylaxis, a phenomenon based on the fact that an animal may become sensitized to proteins to react at a later time to the same protein when it is injected or even eaten. To illustrate, a guinea pig may be given an injection of diluted egg white which sensitizes the animal; another injection after an interval of several days will cause marked symptoms such as paralysis, respiratory difficulties and perhaps death. It is now known that human beings may be sensitized with proteins in the same manner. This reaction is possible after eating certain proteins as well as after receiving injections of protein preparations such as antitoxic sera.

Attempts are often made to determine what protein, to which the subject has become sensitized, is causing the illness. A series of pure proteins has been prepared for this purpose. A cutaneous test is made with these on the flexor surface of the forearm. After cleaning, the area to be used is lightly cut with lines about $\frac{1}{8}$ inch long and $1\frac{1}{2}$ inches apart. Attempts are made not to draw blood. A little of the protein is rubbed into this area. A positive reaction is indicated by a reddened area about the site of inoculation. This shows that the protein used is the one with which the patient is sensitized or anaphylactic. A negative reaction is characterized by practically no change in appearance of the skin about the site of inoculation. In order to illustrate how this test works, one case may be discussed with some detail. This was a case of wheat asthma but will suffice to explain the general facts about all cases of anaphylaxis. The patient suffered from typical symptoms of bronchial asthma. Cutaneous tests, made according to the technic outlined above, indicated that wheat protein was causing the symptoms. The patient stated that the first attack which she could remember, had occurred many years before when she inhaled some flour during baking; since that time the attacks had recurred at frequent intervals. The patient was informed that wheat was the cause of her trouble and advised to refrain from eating foods which contained wheat flour, or handling the flour. The results were said to have been magical. The symptoms disappeared almost immediately. This patient was "desensitized" by being given small injections of wheat protein and brought to a condition where she could eat a moderate amount of wheat bread daily. Finally, she could eat bread and pastries as desired. The above illustration concerns wheat. The same phenomena have been observed with eggs,

strawberries, cheese, and other foods. The desensitization process is not unlike the building up of immunity according to methods discussed later on. If the protein which is objectionable is in an essential food, the problem is more difficult; if it is in a non-essential one it may easily be dropped from the diet. Desensitization is necessary if it must be eaten. In order to simplify the detection of the protein which is causing the trouble, mixtures of eight or ten may be used for the first tests. These mixtures may be used until that one is found which gives a reaction; then, each protein in the mixture must be tested to find the specific protein.

FOODS WHICH ARE NATURALLY POISONOUS

Some foods are naturally poisonous. Compounds which are poisonous for other species, are formed by several species. The poisonous species may, at times, be confused with those which are non-poisonous.

The confusion of poisonous and non-poisonous species of mushrooms has caused many deaths since very early times. This type of food poisoning was one of the earliest to be reported in the writings of the ancients. Many famous people are said to have lost their lives in this manner. The symptoms are, in general, great drowsiness, stupor, pain, and nausea. The poisoning is extremely serious. It has been stated that increases in such deaths usually occur during times when variety in food is difficult to attain. Deaths from poisonous species of mushrooms were more common immediately after the World War.

White snake root is also poisonous for animals. When the plant is eaten by cows the poison may appear in the milk and cause serious illness among those who drink the milk. The poison is not destroyed by heat because plants which have been cooked in the autoclave have caused poisoning. Pasteurization of milk would not, therefore, prevent this poisoning.

Animal products may also be poisonous. Mussels have caused some serious outbreaks of illness. The real cause of the poisoning in this case does not seem to be known. An extensive outbreak at Wilhelms-haven, Germany, in 1885, caused investigations by many chemists and biologists. More recent outbreaks have been reported in the United States which, although not involving a great many cases, have been serious.

FOODS UNDESIRABLE ON ACCOUNT OF DECOMPOSITION

Decomposed foods have always been regarded as poisonous and most people accept without reservation the universal condemnation that is given them. This attitude is often without foundation beyond

the fact that they often exhibit disagreeable odors and such disgusting appearances that they are regarded as dangerous. This has given basis for theories which do not rest on well-established facts. The term "ptomaine poisoning," for instance, is commonly used as a diagnosis for symptoms of illness which appear in the gastro-intestinal tract and which cannot be attributed to other causes.

The present position has been so well stated in an editorial of the Journal of the American Medical Association that it is reproduced here.

The term "ptomain" poisoning has become a cloak for ignorance. Jordan says that "ptomain poisoning is a convenient refuge from etiologic uncertainty." In fact, any acute gastro-intestinal attack resulting from a great variety of causes is apt to be called "ptomain" poisoning. Selmi, in 1873, first used the word ptomain (from *πτῶμα*, a corpse) to include the poisonous products of putrefaction which gave the reaction then looked on as characteristic of vegetable alkaloids. From the time of Selmi, when ptomains were regarded as animal alkaloids, our conception of these substances has changed markedly. The last attempt to give precision to the term was by Vaughan, who defined ptomains as intermediate cleavage products of protein decomposition. Rosenau and his associates at Harvard have been searching in vain for the past year and a half for ptomains that might cause gastro-intestinal or other symptoms. Split products of protein putrefaction are readily isolated. Some of these products have physiologic activity, but none of them thus far have been demonstrated to be poisonous when taken by the mouth. The so-called ptomains isolated and described by Selmi, Nencki, Brieger, Schmiedeburg, Faust and Vaughan were usually obtained from putrid organic matter that had decomposed past the point at which it would be used as food. Furthermore, most of these substances were tested by injecting them subcutaneously or intravenously into animals. Many substances are poisonous when thus introduced parenterally, though they may be harmless by the mouth. Again, many of the so-called ptomains isolated and described have since been shown to contain impurities. Chemists are now seldom confident of the purity of protein fractions, even when obtained in crystalline form. The chemical search for split protein products as the cause of "ptomain" poisoning has practically been abandoned. Most of these split products are amines, which are either not poisonous at all, or no more so than their corresponding ammonia salts. The chemical resemblance between muscarin and cholin has directed the work toward the phosphatids, but thus far this line of research has not helped solve the puzzle of "ptomain" poisoning. Chemists avoid the use of the word ptomains, for the reason that it lacks precision. This is a curious instance of the popular use of a technical term that sounds well, but means little. Only clinicians cling to it as a convenient refuge. Ptomain is a term for chemical substances of uncertain origin, unknown nature, and doubtful existence.

Many of those who read these pages will not like to believe these

statements nor give up opinions which have been formed over years of observation and contact with illnesses of various sorts. Even with danger of repetition and monotony, the author quotes below another editorial from the Journal of the American Medical Association (Vol. 90 (1928), p. 1573), which appeared just as this manuscript was being prepared for the publisher.

A ptomaine has been defined as a basic organic compound that is formed by the action of bacteria on organic matter. It thus is a chemical entity just as the chemical bases known as alkaloids are. However, the term ptomaine includes a wide variety of compounds some of which are not particularly toxic and none of which are specific in the sense that toxins are. Hence we are reminded by Rosenau that bacteria which are in no sense pathogenic may be capable of producing ptomaines, while others which are highly pathogenic may produce few or none of these basic derivatives. The outcome of present-day consideration is that most of the cases of so-called ptomaine poisoning that cannot be attributed to quite independent clearly defined etiologic factors are recognized as infections with certain bacteria, such as those of the paratyphoid group or as intoxications with bacterial toxins such as those of the botulinus organism.

As *The Journal* has pointed out, a clinical diagnosis of food poisoning, especially when it is suspected that the food is contaminated with certain bacteria or their toxins, should be supported by epidemiologic, bacteriologic and toxicologic investigations. The nondescript expression "ptomaine poisoning" should be entirely abandoned. For the most part it is a misnomer; and, as Jordan has stated, it is used to decide an etiologic uncertainty. Illness due to food may arise from bacterial infection of the food, from toxins retained in it, or from a large variety of organic and inorganic contaminants. Infected food is far more harmful than decomposed food, as a rule. Food is at most a vector of harm which may range from a microbe causing enteritis to the poison of a toxic mushroom or the accidental presence of a noxious element like arsenic or mercury. In any event there is no proper place in any of these diverse categories for the expression "ptomaine poisoning." The haphazard diagnosticians will miss the self-satisfying euphony of these words, and the public may regret the passing of the verbal symbol of the mystery of upset "inner workings" of mankind. Nevertheless the plea for the abandonment of an admittedly inconclusive designation of disease must win.

The following reasons may be offered to explain why ptomaine poisoning is not a satisfactory diagnosis in illness.

1. Foods which would cause it are usually in an advanced state of decomposition. A discriminating consumer would not eat foods which exhibited signs of spoilage. This would tend to prevent such illness as ptomaine poisoning. However, this statement only partly represents the truth. Some of the most typical outbreaks of botulism have been caused by foods which were

recognized as "off" in characteristics by the consumers. Some of these foods were prepared by cooks for service to others and it is possible that they might be a little more careless.

2. Certain of our foods are purposely subjected to decompositions not unlike those which take place in decaying foods. These decompositions are carried out to improve the flavor of the food product. Cheese, for instance, is prepared from the casein of different types of milk; it is "ripened" or allowed to undergo bacterial decomposition in order to improve the flavor. Limburger cheese is a typical example of this. It possesses many of the characteristics of putrefaction which are not at all unlike those of decaying meat or other protein. The use of such foods as Limburger cheese complicates diagnoses of ptomaine poisoning. On one hand putrid food is shunned and regarded as poisonous while on the other it is selected. Wild fowl in some countries is allowed to undergo incipient putrefaction. This is called ripening and is supposed to improve the flavor. Such fowl are believed to be more desirable on account of the improvement in flavor and more tender. The American taste does not look with favor on such practices, for fowls handled in this manner are not "fresh." A missionary in Alaska recounted the practice of the natives who bury fish heads until they have reached an advanced state of decomposition when they are dug up and eaten. The Chinese people prepare eggs by storing them in a mixture of lime, salt, and other substances. These eggs are eaten after a lapse of time extending even to several years.

These are several examples of foods which are made to putrefy. If putrid foods cause illness certainly these that have been mentioned should be dangerous.

3. Better reasons may often be found for illnesses which are believed to be ptomaine poisoning. Botulism, in some cases, was called ptomaine poisoning. Reports in the literature of large outbreaks of illness diagnosed as ptomaine poisoning state that investigations by medical experts found better explanations. In two cases arsenical poisoning was found to be the explanation. Bacterial toxins may be present in foods and cause the symptoms called ptomaine poisoning.

4. The symptoms of ptomaine poisoning are too inclusive. Many of the symptoms which are used for the diagnosis of ptomaine poisoning are exhibited by other diseases.

5. The laboratory data on the toxicity of the so-called "ptomaines" have come mainly from injections of these bodies into animals and not feeding. Before a substance formed in the decomposition of proteins can be considered to be significant as a cause of food poisoning, its poisonous properties must be established by feeding and not by injection into the tissues or under the skin.

These statements about ptomaine poisoning are not supposed to justify the eating of old putrid food. Fresh foods produced under clean conditions should be demanded; the above discussion should convince one

that when gastro-intestinal disturbances occur, the real causes should be searched for. Spoiled food should never be eaten.

Storing Foods in the Opened Tin Can.—This topic is worthy of attention since it is one of the fallacies which has been handed down from generation to generation. It is kept alive in various ways. Some food packers for reasons which they find difficult to offer, continue to print the legend on cans of food that the contents should be emptied from the can immediately after opening. It is often said that this practice will prevent ptomaine poisoning. From the standpoint of bacteriology there is just as much danger in eating foods stored in a sterling silver dish, or in expensive chinaware. Our forefathers had practically no other kitchen utensils except those made from tin. Milk was often set away in tin pans for the cream to rise before skimming. The cream was made into butter while the curd was often made into cottage cheese. This type of container caused little worry but the use of a tin container in the shape of a tin can was tabooed. The type of container is of far less importance than the methods under which the containers of food, irrespective of their composition, are handled. The bacteria which gain entrance are the agents to which attention should be directed.

FOODS CONTAINING METALLIC SALTS

The metallic salts which may be contained in foods have been a favorite explanation of illness. Some of the salts of the heavy metals are very poisonous but those generally found in foods are not.

Tin.—Early medical literature contains many references to poisonings by tin salts. Practically all of them were incomplete investigations. Pharmacologists state that tin salts are not poisonous and one even states that chronic tin poisoning is unknown. If tin salts were poisonous, there would certainly be more cases and plenty of opportunity to study tin poisoning in these days when so much canned food is used.

Arsenic Compounds.—Arsenic has been the cause of a number of outbreaks of poisoning. Fruit trees are sprayed with arsenical compounds and if sprayed late enough in the fall the fruit might be objectionable. Horticulturists are quite well agreed that if spraying is done in accordance with good practice there is little danger. Accounts are on record of the contamination of sugar during shipment with arsenical compounds to be used for spraying. It is easy for food to be contaminated with these compounds to cause illness which might be attributed to other causes.

BOTULISM

This is by far the best illustration of food poisoning that can be offered. It is a true toxemia resulting from the eating of food in which *Clostridium botulinum* has grown and formed its poison.

Botulism has received much study in America since 1915 on account of the numerous striking outbreaks that have occurred. It has enjoyed an interest far out of proportion to its significance as a cause of death. The striking symptoms and high fatality gave it an importance beyond its due.

The symptoms of botulism may best be enumerated by quoting the description of a case from the literature. The following case was reported by Dickson in 1918.

Outbreak 12 (one case).—On Friday, Feb. 22, 1918, Mrs. H. of Oakdale, Calif., tasted a small portion of a bean pod from a jar of home-canned string beans which she had just opened. The beans had a peculiar irritating taste but she swallowed a small amount. When she placed the beans on the stove to cook there was an extremely disagreeable odor and she discarded them.

On Saturday morning, February 23, Mrs. H. was dizzy when she first got out of bed and she staggered when she walked. She noted that vision in the left eye was blurred. By afternoon vision was blurred in both eyes and the staggering was much worse. The patient became alarmed and consulted Dr. J. B. Thompson of Oakdale, to whom the writer is indebted for the following report.

When first seen by Dr. Thompson, Mrs. H. was almost hysterical but had no complaint other than that which has been detailed. She said that there had been no pain and no gastro-intestinal disturbance, and that she had not even been nauseated.

Examination showed complete dilatation of both pupils, which reacted very sluggishly to light, and a pulse rate of about 100 per minute. The temperature was normal. The patient was very weak and muscular movements were not coordinated, but nothing else abnormal was found. Dr. Thompson stated that he was strongly suspicious of belladonna poisoning, as at that time the patient denied having eaten any spoiled food.

After midnight, Saturday, Mrs. H. complained that her tongue was swollen, and she began to have difficulty in talking. On Sunday morning there was bilateral ptosis and later in the forenoon she began to have difficulty in swallowing. Speech rapidly became unintelligible. About noon on Sunday she began to vomit and the vomiting continued for the greater part of the afternoon. There were three formed stools during Sunday forenoon and several involuntary liquid stools during the afternoon. All the stools were extremely offensive. There were no bowel movements during the last thirty-six hours of life. Toward evening on Sunday the patient began to have spells of strangling when she attempted to swallow, and she complained bitterly of

the accumulation of mucus in the pharynx, which she was unable to raise. Urination was not disturbed except that the patient voided involuntarily during strangling spells.

The temperature varied from normal to 102.6 (rectal) just before death, respiration remained about 24 and the pulse varied from 98 to 120 per minute.

The patient was very restless and did not sleep during Sunday night or Monday. There was no disturbance of mentality and the skeletal reflexes were intact. There was no disturbance of sensation except a sense of numbness in the hands. She became rapidly weaker and the pulse increased in rapidity. At first there was satisfactory response to stimulation but later there was no appreciable effect. The patient died early Tuesday morning about eighty-eight hours after tasting the beans.

To remove the mucus from the pharynx, Dr. Thompson made use of an aspiration bottle to which a soft rubber catheter was attached. He stated that this appeared to give much greater relief and to cause much less irritation than swabbing.

The beans which caused the poisoning were grown in Oakdale and were, picked but a few hours before they were canned. They were washed, strung, broken into small pieces and boiled in an open kettle for twenty minutes. They were then packed into new, freshly-boiled, 1-pint economy jars, 1 tablespoonful of salt was added to each jar and the jars were covered, immersed into boiling water, which covered them, and boiled actively for three hours. Twelve jars of beans were canned and two of them spoiled. The contents of nine of the others were eaten after they had been cooked, and appeared to be good, but there is no record as to whether any one had eaten or tasted any of the beans before they were cooked. One jar was taken to the laboratory for bacteriologic examination, but no evidence of *B. botulinus* was found.

A portion of the beans from the contaminated jar was gathered from the garden where they had been thrown and had lain in the rain for several days. Bacteriologic examination showed no evidence of *B. botulinus*.

Clostridium botulinum.—This organism was discovered by van Ermengem in a study of an outbreak of illness following the eating of sausage. Having isolated the organism and shown that it formed a powerful poison, he named it from the Latin word for sausage since he isolated it from sausage. It is known to-day that many other foods may contain the poison.

The microorganism possesses three major characteristics which give it importance in food poisoning:

1. It forms a powerful poison which is active even in very small amounts.
2. It forms very resistant spores which survive processing of canned foods.
3. It is a strict anaerobe, thus finding the conditions in a can of food suited to its development.

If one could create an organism he could hardly select characteristics

which would be more significant in food poisoning than these which have just been named.

The toxin formed by *Clostridium botulinum* is one of the most active poisons known. A very small amount will cause death. Some fatal cases have been reported where a little of the liquor left on the tongue from tasting a portion of a bean have caused death with all of the typical symptoms. Experiments have shown that this toxin is quite susceptible to heat and destroyed by boiling for a few minutes. This, then, gives us one of the simplest methods of preventing poisoning from canned foods which have been especially significant in causing botulism.

The soundness of the advice to cook canned foods before they are tasted is well borne out by the following report from the Weekly Bulletin of the California State Board of Health for March, 1923:

A woman residing in Glendale, Los Angeles County, opened a jar of home-packed string beans a few days ago. She tasted the beans, and afterwards cooked them for ten minutes, serving them to the other members of her family consisting of her husband and six children. Three days later this woman developed marked symptoms of botulism—general weakness, disturbed vision and throat paralysis. After suffering acutely for nearly a week, the patient died in great agony. None of the other members of the family suffered any ill effects from eating the beans, the cooking of which undoubtedly destroyed the toxin so that the other members of the family were not affected. Some of the uncooked beans apparently were thrown out to the chickens, for about eight hours after the garbage had been thrown out to the fowls, all of them showed symptoms of limberneck and died.

The family was warned immediately not to eat any more of the home-canned product that remained unopened, and samples of beans from the same pack have been sent to the State Hygienic Laboratory for examination.

The spores are exceptionally resistant to heat, perhaps more resistant than the spores of any other microorganism.¹ This allows the organism to survive processes given canned foods. Estey and Meyer reported an extensive study of the heat resistance of the spores of this organism. They reported the following data on the heat resistance in a phosphate solution of a pH of 7:

4 minutes at 120° C. (248° F.)
10 minutes at 115° C. (239° F.)
32 minutes at 110° C. (230° F.)
100 minutes at 105° C. (221° F.)
330 minutes at 100° C. (212° F.)

It is a very significant fact that in most of the important outbreaks of food poisoning caused by the toxin of *Clostridium botulinum*, the foods

¹ The spores of some of the thermophilic bacteria are probably more resistant.

have been admitted to be abnormal in some way. If the consumers had followed the advice of food experts, poisoning would not have resulted.

FOOD-BORNE INFECTIONS

Infection has been defined as the entrance and growth of a parasite in a host; food-borne infections, then, are infections in which the etiological agent is disseminated by foods. A large number of bacteria may be carried by foods and thus fall into a discussion of food infection. However, the so-called paratyphoid organisms are best known in this respect. The principal members of this group are *Salmonella paratyphi* (*Bacterium paratyphosum A*) and *Salmonella schottmülleri* (*Bacterium paratyphosum B*). These microorganisms may cause infections as well as poisonings. When they cause infections, the cells themselves are disseminated in the foods; when toxemias are caused the toxins formed by the cells are disseminated. In infections an incubation period takes place between the ingestion of the infected food and the appearance of the first symptom; in poisoning, the symptoms appear very soon, usually within a few hours, after the food has been eaten. Several investigations are reported in bacteriological literature showing that these organisms can grow in various common foodstuffs. In some cases the symptoms of illness appeared so quickly after eating that the victims had to leave the table.

Other parasites besides bacteria may be spread by foods. *Trichina spiralis* causing trichinosis is spread by infected uncooked pork.

Salmonella enteritidis.—This organism was isolated by Gaertner in 1888 during an epidemic of food poisoning caused by meat. Nearly sixty persons were afflicted and one died. The symptoms were mainly gastro-intestinal. Gaertner found that the cow from which the meat had been taken had been slaughtered on account of disease. When the microorganism which was isolated in this investigation was fed to laboratory animals, certain of them also developed illness. Before Gaertner described his organism other outbreaks had been reported which were probably caused by the same organism. The characteristics of the organism are not uncommon. It grows well on all laboratory media; evidence is also available to indicate that the organism forms a heat-resistant toxin.

The Paratyphoid Bacilli.—These two microorganisms were isolated from diseases which showed symptoms much like those of typhoid fever. On account of their similarity to the organism causing typhoid fever, they were called "paratyphoid bacilli."

Food Infection by Paratyphoid Bacilli: This type of illness is a true

infection caused by the ingestion of the paratyphoid bacilli. An incubation period is required usually lasting ten or fifteen days. The mortality is quite low, usually less than 2 per cent. The organisms have been spread by such foods as milk, oysters, and the like.

Food Poisoning by Paratyphoid Bacilli.—Unlike the slow development of paratyphoid fever, the poisoning resulting from the paratyphoid bacilli may manifest itself very soon after eating. The symptoms are violent and gastro-intestinal in nature. The toxin seems to be heat-resistant in some cases.

Contamination of Foods by Carriers.—A carrier is an individual who harbors and excretes disease-producing bacteria but who shows no symptoms of illness. Since carriers are discussed in a subsequent chapter we will proceed here to describe one or two carrier-food-borne outbreaks of typhoid fever.

The first one to be mentioned was investigated by Sawyer of the California State Board of Health.

The food was found to have been infected by a typhoid carrier who had no knowledge of ever having the disease. A study of the manner in which the infection reached the food fastened suspicion on a dish of Spanish spaghetti. This dish, which contained a thickening sauce composed chiefly of milk, was prepared by the carrier in her home on the day before the dinner. The baking of the dish was done at the dining-hall during the morning before the meal. As there was ample time for the dish to become infected with the typhoid organisms during its preparation, it was only necessary to prove that the dish was a favorable medium for the growth of the typhoid bacillus and that the final baking of the dish had been insufficient to sterilize it, in order to prove definitely that the spaghetti had been the source of infection. To determine these two points laboratory experiments were conducted which produced valuable data regarding the temperatures reached in baking as carried out by the ordinary household methods.

A dish of spaghetti was prepared in the laboratory under conditions simulating as nearly as possible those under which the original dish had been prepared, and inoculated with a broth culture of the typhoid bacillus of the strain obtained from the carrier. This material, which was 5 in. deep and from 9 to 13 in. in diameter, was baked in the hot oven of an ordinary gas range for fifteen minutes. At the end of this time the temperature in the middle of the spaghetti had risen from 16 to 17° C. and after standing in the room for one-half hour rose to 21° as the heat penetrated to the inner portion. Cultures made from the contents of the dish at various depths, after this baking, all developed colonies of the typhoid bacillus.

The spaghetti was next introduced into a hot-air sterilizer, which had been heated to between 160 and 170°, and was subjected to this temperature for thirty minutes. At the end of that time the appearance of the dish suggested

thorough cooking but the temperature at the top was found to be 54° and at the middle only 23° . Cultures made from the contents of the dish, at various depths, after this baking showed the presence of typhoid bacilli.

The dish of spaghetti was finally introduced into an oven maintained at 207 to 214° and subjected to this temperature for one-half hour. Examination of the dish at the end of this period showed the temperature just beneath the surface of the spaghetti to be 83° , at the middle 28° , and at the bottom 48° . After standing in the room for one hour the temperatures were 46° near the top, 42.5° at the middle, and 43° near the bottom. Cultures taken from the middle of the dish showed an abundance of typhoid bacilli. These results showed conclusively that the baking, which the dish had received after being infected, was not sufficient to produce sterilization.

Portions of the sauce were sterilized, inoculated with the same strain of the typhoid bacillus, and allowed to incubate. A study of the rate of development of the bacteria showed the sauce to be a good culture medium for the typhoid bacillus, although somewhat inferior to sterilized skim milk.

In the opinion of the author the results of this investigation demonstrate that "cooked dishes must be considered as possible conveyers of infection unless it can be shown that the method of cooking would produce complete sterilization." The slowness with which heat penetrates dishes like the Spanish spaghetti shows that very prolonged heating would be necessary for sterilization of large dishes of such food. Ordinary baking merely incubates the interior of these masses of food.

Another interesting example of a carrier who repeatedly infected foods is so-called "Typhoid Mary." She was a cook who refused to cooperate with health authorities and finally had to be taken into custody to prevent her from continuing her occupation as a cook. In 1906 Dr. Soper was called to a summer home on Long Island, New York, to investigate the cause of typhoid fever which had appeared. Six individuals in a household of eleven were afflicted with the disease. Evidence pointed to the cook as the cause but no information could be secured from her when she was found. Dr. Soper was able to show that she had caused seven outbreaks in eight families in which she had worked as a cook. In some of them typhoid fever appeared with marked regularity after Mary Mallon took up her duties as a cook. Repeated attempts were made, without success, to collect specimens of urine, feces and blood for examination. Finally she was forcibly removed to a detention hospital of the New York City Health Department where she was detained virtually as a prisoner for three years. The action of the health department was sustained by the courts when attempts were made to release her. Later, she was voluntarily released on her promise to refrain from cooking. She finally broke her parole and disappeared. For nearly five years she was lost but appeared again under very dramatic circum-

stances. An outbreak of typhoid fever appeared in a hospital in New York City. It was finally traced to the cook who proved to be Typhoid Mary. She was again taken into custody by the New York City Health Department with little prospects of being released. The total known cases were 51 with 10 outbreaks. Only one's imagination will let him estimate the number of unknown cases which she caused.

No more space need be given to carriers although interesting examples could be mentioned almost without end. The Mallon case has lessons for anyone who wishes to ponder over the facts. It shows, first of all, that our foods may easily become contaminated with excrement. Sanitarians state that for a person to have typhoid fever, he must swallow some of the excrement from a person ill with the disease. Another lesson is that cooks should be selected with great care. Their past history should be known, at least as far as their disease record is concerned.

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CHAPTER XXVI

RELATION OF BACTERIA TO DISEASE

The relationship of bacteria to disease is interesting. Unfortunately, the best known facts about bacteria are their relations to certain diseases. The layman, who may not have been trained in microbiology, often places all bacteria into this unwelcome group. In former chapters we have discussed some of the useful applications of microorganisms to everyday life and have learned that many of them are willing to work for man if given the proper conditions.

What is Disease?—The term disease is not an easy one to define. It may be one of those terms the definition of which should not be attempted in a few words. It is profitable to analyze some of the different conceptions of it. Some regard disease as parasitism, the infected organism being the host and the etiologic agent being the parasite. It will be unnecessary to discuss parasitism now for it has been taken up in a former chapter. As stated a few paragraphs on in this chapter, it is difficult to distinguish between a parasite and a saprophyte. An organism may exist on a host for a long time without causing illness and eventually cause symptoms of disease when conditions are favorable. Consequently, if disease is regarded as a case of parasitism, some distinction may have to be made between an organism which is living on a host without causing illness and one which has become aggressive enough to cause disease.

Disease may also be regarded as a conflict between two opposing forces. This conception has some merit. It allows one to correlate many general statements which have been advanced to explain freedom from disease. If disease is a conflict, the outcome will depend on the strength of the invading organism and the defensive powers of the host. We have long been advised to keep up resistance by proper living habits, good food, fresh air, etc., if we wish to be free from disease. The size of the dose or in this case the number of microorganisms often determines whether a person will be infected or not. A massive infection often overcomes a grade of resistance to disease which would ordinarily be sufficient to protect.

THEORIES OF DISEASE

Since early times many different theories have been proposed to explain the causes of disease. Some of them seem quite reasonable when analyzed from the standpoint of the data that were available at that time. Others were merely convenient and lacked little foundation either in experiment or observation.

The Demonic Theory of Disease.—This was probably the first explanation of disease. It was propounded before any serious attempt was made to determine the real causes. According to the demonic theory, disease was due to the presence of evil spirits or demons in those who were afflicted. The treatment was often just as unreasonable as the diagnosis. It frequently consisted in making great noise around the patient so that the devils would depart. Certain articles were worn to keep away evil spirits. Remnants, if not large portions of these early theories, have come down through the ages to the present day. During epidemics of disease, it is not uncommon to see individuals wearing little bags of drugs about their necks; other practices such as the wearing of fetishes, painting themselves, etc., are commonly practiced even by informed people to-day. Such practices, however, seem ridiculous when thought of in connection with so-called savage people.¹

Humoral Theory.—The humoral theory was used by the Greeks for explaining the causes of disease. According to this theory there are, in the body, four humors: blood, mucous or phlegm, black bile and yellow bile. In a state of health these four humors are present in equal concentration but in disease some one predominates. From the information which medical men had in those days, this theory had some merit. Accordingly, when certain symptoms appeared which were thought to indicate the presence of too much blood, the patient was bled. Colds would be explained on the basis of too much phlegm.

Germ or Zymotic Theory.—In the first chapter the history of bacteriology was reviewed and some consideration was given to the discussions and experimental work which led up to the germ theory of fermentation. After that theory had been proven it was not a long step to apply the facts to disease. This resulted in the germ theory of disease. The analogy between a typical fermentation and a case of infectious disease was strikingly shown by Sedgwick as follows:

¹ Some idea of the present status of this theory may be secured by consulting the following paper: African Aboriginal Therapy, by P. A. E. Sheppard, *American Jour. Pub. Health*, **10** (1290) 227-235.

A Fermentation
(Apple Juice)

1. Exposure of juice to air, dust, etc.
2. Repose and then slow change.
(Growth of the ferment)
3. Active fermentation or "working." Evolution of gas bubbles, change of sugar to alcohol.
Rise of temperature
4. Gradual cessation of fermentation
5. No further liability to alcoholic fermentation

An Infectious Disease
(Smallpox)

1. Exposure of patient to infection
2. Incubation. (Slow and insidious progress of the disease)
3. Active disease. Eruption, disturbance of the usual functions.
Rise of temperature
4. Slow convalescence (or death)
5. Immunity to smallpox

This contrast of smallpox with a fermentation shows the marked similarity between the two. As soon as Pasteur had shown that fermentation was a biological phenomenon and that the silkworm disease was caused by a microorganism, the germ theory of disease was firmly established. Other facts were soon gathered to support it.

Zymotoxic Theory.—The germ theory did not explain all of the facts which were soon discovered. It did not explain, for instance, the symptoms of illness which result from the presence of toxins. Consequently, the germ or zymotic theory was extended and became the zymotoxic theory. Bacterial toxins are discussed at greater length later in this book. They furnish the basis for the zymotoxic theory of disease.

TYPES OF DISEASE

For convenience, diseases have been divided into groups. Certain terms have slowly crept into use. Some of these we will review. Diseases are often spoken of as acute or chronic. An acute disease is one which reaches its greatest intensity or crisis quickly. Such diseases are typhoid fever, diphtheria, tetanus, etc. Conversely, a chronic disease progresses slowly and may extend over months and years. Rheumatism is perhaps a good example. On the other hand, a disease like tuberculosis may be either chronic or acute depending on the conditions. Diseases are also classed as endemic, epidemic and pandemic diseases. A disease is endemic when it is constantly present in a small number of cases. Diphtheria is often endemic. When an endemic disease increases or flares up, so to speak, within a short time and a restricted area, it becomes epidemic. A pandemic disease is a widespread epidemic. Influenza is a good example of a pandemic disease. During 1918, it not only spread over whole nations but the whole world was more or less infected.

Contagious, Infectious and Communicable Diseases.—These three adjectives are used in much the same manner. A contagious disease is one which may be spread by mediate or immediate contact. An infectious disease is one which is caused by some living parasite such as a bacterium, protozoan or fungus; it may or may not be contagious. These are the definitions given by one standard dictionary of medical terms. These terms are not as satisfactory as once believed. In many cases absurd distinctions were made between diseases. On account of this, some present-day sanitarians have suggested the substitution of the term *communicable* for the older terms *contagious* and *infectious*. The former is undoubtedly a better term because it does not bring unnecessary restrictions but emphasizes only that characteristic in which the sanitarian is interested.

Diseases of Known and Unknown Etiology.—Not all diseases and illness are caused by microorganisms. Those which are supposed to be, are divided into two groups. One group is composed of those diseases which are said to be of known etiology, i.e., the causal organism is known and can be isolated in pure culture. With these diseases great progress has been made in the study of methods of treatment and cure. The other group is composed of diseases of unknown etiology in which the causal organism, or etiologic agent, is unknown. The question may arise in the reader's mind how we are certain that diseases of unknown etiology are caused by microorganisms. The methods of dissemination and communication in such diseases indicate that some living virus is probably the cause. The etiologic agent, for instance, of smallpox is unknown but the characteristics of the disease indicate that some living agent is involved.

Koch's Postulates.—No bacterium can be said to cause a disease until certain facts have been proven. These prerequisites were stated as early as 1840 by Henle. On account of the uncertainty of pure cultures, they may not have been adequately tested by Henle. However, Koch was able to fulfill them for the tuberculosis organism. Since then they have been known as Koch's postulates, canons or laws. They may be stated as follows:

1. The microorganism must be present in all cases of the disease.
2. It must be isolated from the infected animal and grown in pure culture.
3. The original disease must be reproduced with the microorganism when it is injected into a susceptible animal.
4. In this case of the disease the organism must be found as in the original case.

Before a microorganism may be indicated as the causal agent in any dis-

1. Food - N. K. K. K.
2. Buffer

TYPES OF DISEASE

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TABLE VIII
DISEASES OF KNOWN ETIOLOGY

Disease	Infectious Agent	Date of Discovery	Discoverers	Habitat
Anthrax.....	Bacillus anthracis	1876	Koch	Animals and man with anthrax
Cerebrospinal meningitis.....	Neisseria intracellularis	1887	Weichselbaum	Cause of epidemic meningitis
Cholera.....	Vibrio comma	1884	Schröter	Intestinal contents of cholera patients
Diphtheria.....	Corynebacterium diphtheriae		Klebs-Löffler	Cause of diphtheria in man
Dysentery (Bacillary).....	Eberthella dysenteriae	1898	Shiga	Cause of dysentery in man
Glanders.....	Pfeifferella mallei	1886	Löffler	Cause of glanders
Gonorrhea.....	Neisseria gonorrhoeae	1879	Neisser	Cause of gonorrheal infection
Influenza.....	Hemophilus influenzae	1892	Pfeiffer	Said to cause influenza by Pfeiffer
Leprosy.....	Mycobacterium leprae	1879	Hansen	Cause of leprosy
Malaria.....	Plasmodium vivax	1880	Laveran	
Paratyphoid fever.....	Salmonella schottmülleri	1900	Schottmüller	Cause of paratyphoid fever
Plague.....	Pasteurella pestis	1894	Yersin and Kitasato	Cause of plague
Pneumonia (Lobar).....	Diplococcus pneumoniae	1886	Weichselbaum	Cause of lobar pneumonia
Rocky Mountain spotted fever	Rickettsia bodies		Various investigators	Throats of persons ill with scarlet fever
Scarlet fever.....	Streptococcus scarlatinae			
Septic sore throat.....	Hemolytic streptococcus			Cause of septic sore throat
Syphilis.....	Treponema pallidum	1905	Schaudinn	Cause of syphilis
Tetanus.....	Clostridium tetani	1884	Nicolaier	Cause of tetanus (lockjaw)
Tuberculosis.....	Mycobacterium tuberculosis	1884	Koch	Cause of human tuberculosis
Typhoid fever.....	Eberthella typhi	1880	Eberth	Cause of typhoid fever
Whooping cough.....	Hemophilus pertussis		Bordet and Gougou	Cause of whooping cough
Yellow fever.....	Leptospira icteroides	1919	Noguchi	Cause of yellow fever

DISEASES OF UNKNOWN ETIOLOGY

Chicken pox	Rabies
Dengue	Smallpox
German measles	Typhus fever
Measles	
Mumps	
Polomyelitis	

Slavonia T. in m. a. b. a. m. e. d. i. a. m. o. r. e. a. i. c. a.

state media to make possible either

ease, these requirements should be fulfilled. However, there are difficulties in fulfilling them in some diseases even though the causal organism is quite well established. Such a situation may exist with typhoid fever. A number of accidental infections of human beings, however, gives sufficient experimental foundation to show the relation of *Eberthella typhi* to typhoid fever.

Use of Animals in Experimental Work in Bacteriology.—The fulfilment of Koch's postulates and other work in bacteriology require the use of animals. Such animals as guinea pigs, rabbits, rats, monkeys, mice, etc., are more commonly used. Human beings have been used at times and it is not difficult to secure human volunteers for experiments. Such was the case during the classic investigations by Reed, Carroll, Agramonté and Lazear on the relation of the mosquito to the dissemination of the organism causing yellow fever. A sufficient number of privates in the American Army offered themselves as subjects for experimentation; they were bitten by mosquitoes which had bitten patients suffering from yellow fever. Experiments on leprosy and scarlet fever have also been made on human subjects who offered themselves for the sake of truth. Advances in medicine make experiments on living organisms necessary. The propriety of such animal experimentation is being discussed to-day about the term *vivisection*. An enlightening article in vivisection is just from the pen of H. G. Wells. One paragraph might be quoted:

What is vivisection? It is a clumsy and misleading name for experimentation upon animals for the sake of the knowledge to be gained thereby. It is clumsy and misleading because it means literally cutting up alive, and trails with it to most uninstructed minds a suggestion of highly sensitive creatures bound and helpless, being slowly anatomized to death. This is an idea naturally repulsive to gentle and kindly spirits, and it puts an imputation of extreme cruelty upon vivisection which warps the discussion from the outset.

It is well known by those who are familiar with the methods used in animal experimentation that there is practically no "vivisection" in animal experimentation in the popular sense. Anaesthetics are used when there is any cutting. The arguments of the anti-vivisectionists are centered about cruelty and pain. They overlook various other practices which may be more painful to animals than laboratory experimentation. Wells called attention to the lives of "fancy" dogs, invalid and grotesque deformations of the canine type, which must be uncomfortable beyond all comparison with the condition of creatures inoculated by the physiologist.

This is a question on which the student should think his way through. It is an important topic of discussion to-day. The whole argument goes down to the question whether a human life is worth the cost in animal experimentation. Many of those who are reading this paragraph would not be alive to-day if it had not been for the availability of diphtheria antitoxin, or antitoxins of other types. These are made in the blood streams of healthy animals.

Characterization of Common Diseases.—A very useful summary of information about forty-two of the more common diseases has been prepared by a Committee of the American Public Health Association and officially approved by the United States Public Health Service.² Parts of this report which deal with diseases caused by bacteria should be of interest to students of bacteriology; they are reproduced herewith.

Produce spores on *medic* | But not in body—
ANTHRAX

1. *Infectious agent.*—Anthrax bacillus, *Bacillus anthracis*.
2. *Source of infection.*—Hair, hides, flesh, and feces of infected animals.
3. *Mode of transmission.*—Inoculation as by accidental wound or scratch, inhalation of spores of the infectious agent, and ingestion of insufficiently cooked infected meat.
4. *Incubation period.*—Within seven days.
5. *Period of communicability.*—During the febrile stage of the disease and until lesions have ceased discharging. Infected hair and hides of infected animals may communicate the disease for many months after slaughter of the animal, and after curing of hide, fur, or hair, unless disinfected.
6. *Methods of control:*

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by bacteriological examination.
2. Isolation of the infected individual until the lesions have healed.
3. Immunization—None.
4. Quarantine—None.
5. Concurrent disinfection of the discharges from lesions and articles soiled therewith.
6. Terminal disinfection—Thorough cleaning.

(B) General measures—

1. Animals ill with a disease presumably anthrax should be placed immediately in the care of a veterinary surgeon. Proved animal cases of the disease should be killed promptly and the carcasses destroyed, preferably by fire.
2. Isolation of all animals affected with the disease.

² Emerson H., (Chairman) et al., *The Control of Communicable Diseases*. Public Health Reports **41** (1926), 2895-2929. Also In the *American Journal of Public Health* **17** (1927), Supplement to the March Issue.

Supplied by

3. Immunization of exposed animals under direction of Federal or State Department of Agriculture.
4. Post-mortem examinations should be made only by a veterinary surgeon, or in the presence of one.
5. Milk from an infected animal should not be used during the febrile period.
6. Control and disinfection of effluents and trade wastes and of areas of land polluted by such effluents and wastes from factories or premises, where spore-infected hides or other infected hide and hair products are known to have been worked up into manufactured articles.
7. A physician should be constantly employed by every company handling rawhides, or such companies should operate under the direct supervision of a medical representative of the health department.
8. Every employee handling rawhides, hair, or bristles who has an abrasion of the skin should immediately report to a physician.
9. Special instruction should be given to all employees handling rawhides in regard to the necessity of personal cleanliness.
10. Tanneries and woolen mills should be provided with proper ventilating apparatus so that dust can be promptly removed.
11. Disinfection of hair, wool, and bristles of animals originating in known infected centers before they are used or assorted.
12. The sale of hides from an animal infected with anthrax should be prohibited. A violation of this regulation should be immediately reported to the state commissioner of agriculture, by telegram, stating the time, place, and purchaser to whom the hide was sold. The report should also be sent to the person purchasing the hide. Carcasses should be disposed of under the supervision of the state department of agriculture. The inspection and disinfection of imported hides are under the supervision of the United States Bureau of Animal Industry. In the event that infection is introduced the state agricultural authorities have jurisdiction over infected animals and the local or state health authorities have jurisdiction over infected persons.

CHICKEN POX

1. *Infectious agent*.—Unknown.
2. *Source of infection*.—The infectious agent is presumably present in the lesions of the skin and of the mucous membranes; the latter appearing early and rupturing as soon as they appear, render the disease communicable early, that is, before the exanthem is in evidence.
3. *Mode of transmission*.—Directly from person to person; indirectly through articles freshly soiled by discharges from an infected individual.
4. *Incubation period*.—Two to three weeks.
5. *Period of communicability*.—Until the primary scabs have disappeared from the mucous membranes and the skin.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms. The chief public health importance of this disease is that cases thought to be chicken pox in persons over 15 years of age, or at any age during an epidemic of smallpox, are to be investigated to eliminate the possibility of their being smallpox.

2. Isolation—Exclusion of patient from school, and prevention of contact with non-immune persons.
3. Immunization—None.
2. Quarantine—None.
5. Concurrent disinfection of articles soiled by discharges from lesions.
6. Terminal disinfection—Thorough cleaning.

(B) General measures—None.

CHOLERA

1. *Infectious agent*.—Cholera vibrio, *Vibrio comma*.
2. *Source of infection*.—Bowel discharges and vomitus of infected persons, and feces of convalescent or healthy carriers. Ten per cent of contacts may be found to be carriers.
3. *Mode of transmission*.—By food and water polluted by infectious agent; by contact with infected persons, carriers, or articles freshly soiled by their discharges; by flies.
4. *Incubation period*.—One to five, usually three days, occasionally longer if the healthy carrier stage, before development of symptoms, is included.
5. *Period of communicability*.—Usually 7 to 14 days or longer and until the infectious organism is absent from the bowel discharges.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by bacteriological examination.
2. Isolation of patient in hospital or screened room.
3. Immunization by vaccination may be of value.
4. Quarantine—Contacts for five days from last exposure, or longer if stools are found to contain the cholera vibrio.
5. Concurrent disinfection—Prompt and thorough disinfection of the stools and vomited matter. Articles used by and in connection with the patient must be disinfected before removal from the room. Food left by the patient should be burned.
6. Terminal disinfection—Bodies of those dying from cholera should be cremated if practicable, or, otherwise, wrapped in a sheet wet with disinfectant solution and placed in water-tight caskets. The room in which a sick patient was isolated should be thoroughly cleaned and disinfected.

(B) General measures—

1. Right personal prophylaxis of attendants by scrupulous cleanliness, disinfection of hands each time after handling patient or touching articles contaminated by dejecta, the avoidance of eating or drinking anything in the room of the patient, and the prohibition of those attendant on the sick from entering the kitchen.
2. The bacteriological examination of the stools of all contacts to determine carriers. Isolation of carriers.
3. Water should be boiled, if used for drinking or toilet purposes, or if used in washing dishes or food containers, unless the water supply is adequately protected against contamination or is so treated, as by chlorination, that the cholera vibrio cannot survive in it.

4. Careful supervision of food and drink. Where cholera is prevalent, only cooked foods should be used. Food and drink after cooking or boiling should be protected against contamination, as by flies and human handling.

(C) Epidemic measures—

Inspection service for early detection and isolation of cases; examination of persons exposed in infected centers for detection of carriers, with isolation or control of carriers; disinfection of rooms occupied by the sick, and the detention, in suitable camps for five days, of those desirous of leaving for another locality. Those so detained should be examined for detection of carriers.

DIPHTHERIA

1. *Infectious agent*.—Diphtheria bacillus, *Corynebacterium diphtheriae*, the Klebs-Loeffler bacillus.
2. *Source of infection*.—Discharges from diphtheritic lesions of nose, throat, conjunctiva, vagina, and wound surfaces. Secretions from the nose and throat of carriers of the bacillus.
3. *Mode of transmission*.—Directly by personal contact, indirectly by articles freshly soiled with discharges, or through infected milk or milk products.
4. *Incubation period*.—Usually two to five days, occasionally longer if a healthy carrier stage precedes the development of clinical symptoms.
5. *Period of communicability*.—Until virulent bacilli have disappeared from the secretions and the lesions. The persistence of the bacilli after the lesions have healed is variable. In fully three-fourths of the cases they disappear within two weeks. In 95 per cent of cases, the bacilli disappear in four weeks. In exceptional cases virulent bacilli remain in the throat and discharges for from two to six months.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—By clinical symptoms with confirmation by bacteriological examination of discharges.
2. *Isolation*—Until two cultures from the throat and two from the nose, taken not less than twenty-four hours apart, fail to show the presence of diphtheria bacilli. Isolation may be terminated if persistent diphtheria bacilli prove avirulent. Where termination by culture is impracticable, cases may be terminated with fair safety as a rule sixteen days after onset of the disease.
3. *Immunization*—Exposed susceptibles who cannot be kept under daily observation by a physician or nurse should be promptly immunized by antitoxin. (By susceptibles is meant such individuals as are found to be nonimmune by the Schick test, i.e., those who give a positive reaction.)
4. *Quarantine*—All exposed persons until shown by bacteriological examination not to be carriers.
5. *Concurrent disinfection of all articles which have been in contact with the patient and all articles soiled by discharges from the patient.*
6. *Terminal disinfection*—At the end of the illness, thorough airing and sunning of the sick room, with cleaning or renovation.

(B) General measures—

1. Pasteurization of milk supply.
2. Application of the Schick test to all especially exposed persons, such as nurses and physicians, and active immunization of all susceptibles, but not within three weeks after the administration of antitoxin.
3. **Active immunization of all children by the end of the first year without prior Schick testing; active immunization of school children with or without prior use of the Schick test.**
4. Determination of presence or absence of carriers among contacts and, so far as practicable, in the community at large.

DYSENTERY (BACILLARY)

1. *Infectious agent*.—Dysentery bacillus, *Erberthella dysenteriae*, *Erberthella para dysenteriae*.
2. *Source of infection*.—The bowel discharges of infected persons.
3. *Mode of transmission*.—By drinking contaminated water, by eating infected foods, and by hand-to-mouth transfer of infected material; from objects soiled with discharges of an infected individual or of a carrier; by flies.
4. *Incubation period*.—Two to seven days.
5. *Period of communicability*.—During the febrile period of the disease and until the organism is absent from the bowel discharges.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease.—Clinical symptoms, confirmed by serological and bacteriological tests.
2. Isolation.—Infected individuals during the communicable period of the disease.
3. Immunization.—Vaccines give considerable immunity. Owing to severe reactions their use is not universal, nor should it be made compulsory except under extreme emergency.
4. Quarantine.—None.
5. Concurrent disinfection.—Bowel discharges.
6. Terminal disinfection.—Cleaning.

(B) General measures—

1. Rigid personal prophylaxis of attendants upon infected persons.
2. No milk or food for human consumption should be sold from a place occupied by a patient unless the persons engaged therein occupy quarters separate from the house where the patient is sick, and all utensils used are cleaned and kept in a separate building and under a permit from the health officer.
3. All attendants upon persons affected with this disease should be prohibited from having anything to do with the handling of food.
4. Necessary precautions against flies.
5. Careful supervision of food and drink. Where dysentery is prevalent, only cooked foods should be used. Food and drink after cooking or boiling should be protected against contamination, as by flies and human handling.

GONORRHEA

1. *Infectious agent*.—*Gonococcus*, *Neisseria gonorrhoeae*.
2. *Source of infection*.—Discharges from lesions of inflamed mucous membranes and glands of infected persons, viz., urethral, vaginal, cervical, conjunctival mucous membranes, and Bartholin's or Skene's glands in the female, and Cowper's and the prostate glands in the male.
3. *Mode of transmission*.—By direct personal contact with infected persons, and indirectly by contact with articles freshly soiled with the discharges of such persons.
4. *Incubation period*.—One to eight days, usually three to five days.
5. *Period of communicability*.—As long as the gonococcus persists in any of the discharges, whether the infection be an old or a recent one.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by bacteriological examination or serum reaction.
2. Isolation—When the lesions are in the genito-urinary tract, exclusion from sexual contact, and when the lesions are conjunctival, exclusion from school or contact with children, as long as the discharges contain the infecting organism.
3. Immunization—None.
4. Quarantine—None.
5. Concurrent disinfection—Discharges from lesions and articles soiled therewith.
6. Terminal disinfection—None.

(B) General measures—

1. Education in matters of sexual hygiene, particularly as to the fact that continence in both sexes at all ages is compatible with health and normal development.
2. Provision for accurate and early diagnosis, and treatment in hospitals and dispensaries of infected persons, with consideration for privacy of record and provision for following cases until cured.
3. Repression of prostitution by use of police power and control of use of living premises.
4. Restriction of sale of alcoholic beverages.
5. Restrictions of advertising of services or medicines for the treatment of sex diseases, etc.
6. Elimination of common towels and toilet articles from public places.
7. Use of prophylactic silver solution in the eyes of the new born.
8. Exclusion of persons in the communicable stage of the disease from participation in the preparing and serving of food.
9. Personal prophylaxis should be advised to those who expose themselves to opportunity for infection.

INFLUENZA

1. *Infectious agent*.—Undetermined
2. *Source of infection*.—Probably discharges from the mouth and nose of infected persons and articles freshly soiled with such discharges.
3. *Mode of transmission*.—Believed to be by direct contact, by droplet infection or by articles freshly soiled with discharges of the nose and throat of infected persons.
4. *Incubation period*.—Short, usually twenty-four to seventy-two hours.
5. *Period of communicability*.—Undetermined, apparently during the febrile period or at least for seven days from onset of clinical symptoms.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—By clinical symptoms only. Uncertain in inter-epidemic periods.
2. Isolation—During acute stage of disease.
3. Immunization—None; vaccines have not proved of definite value.
4. Quarantine—None.
5. Concurrent disinfection—Discharges from the nose and throat of the patient.
6. Terminal disinfection—Airing and cleaning.

(B) General measures—

During epidemics efforts should be made to reduce opportunities for direct-contact infection, as in crowded halls, stores, and street cars. Kissing, the use of common towels, glasses, eating utensils, or toilet articles should be avoided. The hands should be washed carefully before eating. In isolated towns and institutions, infection has been delayed and sometimes avoided by strict exclusion of visitors from already infected communities. The closing of schools has not been effective in checking the spread of infection. The use of masks by nurses and other attendants has proved of value in preventing infection in hospitals. Scrupulous cleanliness of dishes and utensils used in preparing and serving food in public eating places should be required, including the subjection of all such articles to disinfection in hot soapsuds. In groups which can be brought under daily professional inspection, the isolation of early and suspicious cases of respiratory tract inflammation, particularly when accompanied with a rise in temperature, may be relied upon to delay the spread of the disease. To minimize the severity of the disease and to reduce mortality, patients should go to bed at the beginning of an attack and not return to work without the approval of their physician.

LEPROSY

1. *Infectious agent*.—Leprosy bacillus, *Mycobacterium leprae*.
2. *Source of infection*.—Discharges from lesions.
3. *Mode of transmission*.—By close, intimate, and prolonged contact with infected individuals. Flies and other insects may be mechanical carriers.
4. *Incubation period*.—Prolonged, undetermined.

5. *Period of communicability*.—Infectivity exist throughout the duration of the disease.

Where good standards of personal hygiene prevail, this disease is but slightly communicable.

6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by bacteriological examination.
2. Isolation for life in national leprosarium when this is possible, or at least until treatment has brought about a healing of all lesions of skin and mucous membranes and the patient has been observed with the disease in this arrested form for not less than six months.
3. Immunization—None.
4. Quarantine—None.
5. Concurrent disinfection—Discharges and articles soiled with discharges.
6. Terminal disinfection—Thorough cleansing of living premises of the patient.

(B) General measures—

1. Lack of information as to the determining factors in the spread and communication of the disease makes any but general advice in matters of personal hygiene of no value.
2. As a temporary expedient lepers may be properly cared for in local hospitals, or if conditions of the patient and his environment warrant, he may be allowed to remain on his own premises under suitable regulations.

MEASLES

1. *Infectious agent*.—Unknown.
2. *Source of infection*.—Buccal and nasal secretions of an infected individual.
3. *Mode of transmission*.—Directly from person to person; indirectly through articles freshly soiled with the buccal and nasal discharges of an infected individual. The most easily transmitted of all communicable diseases.
4. *Incubation period*.—About ten days.
5. *Period of communicability*.—During the period of catarrhal symptoms and until the cessation of abnormal mucous membrane secretions—minimum period of nine days; from four days before to five days after the appearance of the rash.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms. Special attention to rise of temperature, Koplik spots and catarrhal symptoms in exposed individuals.
2. Isolation—During period of communicability.
3. Immunization—By the use of the serum or whole blood of convalescent measles patients, or of any healthy adults who have had measles, given within five days after exposure to a known case of measles, the attack in the exposed person may be averted in a high percentage of instances; if not averted, the disease is modified. Given later, but at a time prior to the clinical onset of the disease, convalescent serum usually modifies

the severity of the attack and the patient acquires the usual lasting immunity to the disease.

4. Quarantine—Exclusion of exposed susceptible school children and teachers from school until fourteen days from last exposure. This applies to exposure in the household. Exclusion of exposed susceptible children from all public gatherings for the same period.
5. Concurrent disinfection—All articles soiled with the secretions of the nose and throat.
6. Terminal disinfection—Thorough cleaning.

(B) General measures—

1. Daily examination of exposed children and of other possibly exposed persons. This examination should include record of the body temperature. A nonimmune⁹ exposed individual exhibiting a rise of temperature of 0.5° C. or more should be promptly isolated pending diagnosis.
2. Schools should not be closed or classes discontinued where daily observation of the children by a physician or nurse is provided for.
3. Education as to special danger of exposing young children to those exhibiting acute catarrhal symptoms of any kind.
4. In institutional outbreaks immunization with convalescent serum of all minor inmates who have not had measles is of value in checking the spread of infection and in reducing mortality.

MENINGOCOCCUS MENINGITIS

1. *Infective agent*.—Meningococcus; *Neisseria intracellularis*.
2. *Source of infection*.—Discharges from the nose and mouth of infected persons. Clinically recovered cases, and healthy persons who have never had the disease but have been in contact with cases of the disease or other carriers, act as carriers and are commonly found, especially during epidemics. Such healthy carriers are not uncommonly found independent of epidemic prevalence of the disease.
3. *Mode of transmission*.—By direct contact with infected persons and carriers, and indirectly by contact with articles freshly soiled with the nasal and mouth discharges of such persons.
4. *Incubation period*.—Two to ten days, commonly seven. Occasionally for longer periods when a person is a carrier for a time before developing the disease.
5. *Period of communicability*.—During the clinical course of the disease and until the specific organism is no longer present in the nasal and mouth discharges of the patient. The same applies to healthy carriers so far as affects persistence of infectious discharges.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by the microscopic and bacteriological examination of the spinal fluid, and by bacteriological examination of nasal and pharyngeal secretions.
2. Isolation of infected persons until fourteen days after onset of the disease.
3. Immunization by the use of vaccines is still in the experimental stage.
4. Quarantine—None.

5. Concurrent disinfection of discharges from the nose and mouth and of articles soiled therewith.
6. Terminal disinfection—Cleaning.

(B) General measures—

1. Search for carriers among families and associates of recognized cases by bacteriological examination of posterior nares of all contacts.
2. Education as to personal cleanliness and necessity of avoiding contact and droplet infection.
3. Prevention of overcrowding such as is common in living quarters, transportation conveyances, working places, and places of public assembly in the civilian population, and in inadequately ventilated closed quarters in barracks, camps, and ships among military units.

(C) Epidemic measures—

1. Increase the separation of individuals and the ventilation in living and sleeping quarters for such groups of people as are especially exposed to infection because of their occupation or some necessity of living conditions. Bodily fatigue and strain should be minimized for those especially exposed to infection.
2. Carriers should be quarantined until the nasal and pharyngeal secretions are proved by bacteriological examination to be free from the infecting organism.

MUMPS

1. *Infective organism.*—Unknown.
2. *Source of infection.*—Secretions of the mouth and possibly of the nose.
3. *Mode of transmission.*—By direct contact with an infected person or with articles freshly soiled with the discharges from the nose or throat of such infected person.
4. *Incubation period.*—From twelve to twenty-six days. The most common period, eighteen days, accepted as usual. A period of twenty-one days is not uncommon.
5. *Period of communicability.*—Unknown, but assumed to persist until the parotid gland has returned to its normal size.
6. *Methods of control:*

(A) The infected individual and his environment—

1. Recognition of the disease—Inflammation of Stenson's duct may be of assistance in recognizing the early stage of the disease. The diagnosis is usually made on swelling of the parotid gland.
2. Isolation—Separation of the patient from nonimmune children and exclusion of the patient from school and public places for the period of presumed infectivity. (See 5.)
3. Immunization—None.
4. Quarantine—None. Exposed susceptible persons should be regularly inspected for the onset, the presence of initial symptoms of the disease, such as fever, or swelling or pain of the parotid or adjacent lymph glands, for three weeks from the date of last exposure.

5. Concurrent disinfection—All articles soiled with the discharges from the nose and throat of the patient.
6. Terminal disinfection—None.

(B) General measures—
None.

PARATYPHOID FEVER

1. *Infectious agent*.—Paratyphoid bacillus A or B; *Salmonella paratyphi*; *Salmonella schottmülleri*.
2. *Source of infection*.—Bowel discharges and urine of infected persons, and foods contaminated with such discharges of infected persons or of healthy carriers. Healthy carriers may be numerous in an outbreak.
3. *Mode of transmission*.—Directly by personal contact; indirectly by contact with articles freshly soiled with the discharges of infected persons or through milk, water, or food contaminated by such discharges.
4. *Incubation period*.—Four to ten days; average, seven days.
5. *Period of communicability*.—From the appearance of prodromal symptoms; throughout the illness and relapses, during convalescence, and until repeated bacteriological examination of discharges show absence of the infecting organism.
6. *Methods of control*:

4) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by specific agglutination test, and by bacteriological examination of blood, bowel discharges, or urine.
2. Isolation—In fly-proof room, preferably under hospital conditions, of such cases as cannot command adequate sanitary environment and nursing care in their homes.
3. Immunization of exposed susceptibles.
4. Quarantine—None.
5. Concurrent disinfection—Disinfection of all bowel and urinary discharges and articles soiled with them.
6. Terminal disinfection—Cleaning.

(B) General measures—

1. Protection and purification of public water supplies.
2. Pasteurization of public milk supplies.
3. Supervision of other food supplies and of food handlers.
4. Prevention of fly breeding.
5. Sanitary disposal of human excreta.
6. Extension of immunization by vaccination as far as practicable.
7. Supervision of paratyphoid carriers and their exclusion from the handling of foods.
8. Systematic examination of fecal specimens, from those who have been in contact with recognized cases, to detect carriers.
9. Exclusion of suspected milk supplies pending discovery of the personal or other cause of contamination of the milk.
10. Exclusion of water supply, if contaminated, until adequately treated with hypochlorite or other efficient disinfectant, or unless all water used for toilet, cooking, and drinking purposes is boiled before use.

RABIES

1. *Infectious agent*.—Unknown.
2. *Source of infection*.—Saliva of infected animals, chiefly dogs.
3. *Mode of transmission*.—Inoculation with saliva of infected animals through abrasion of skin or mucous membrane, almost always by bites or scratches.
4. *Incubation period*.—Usually two to six weeks. May be prolonged to six months or even longer.
5. *Period of communicability*.—For fifteen days in the dog (not known in man) before the onset of clinical symptoms and throughout the clinical course of the disease.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by the presence of Negri bodies in the brain of an infected animal, or by animal inoculations with material from the brain of such infected animal.
2. Isolation—None if patient is under adequate medical supervision, and the immediate attendants are warned of possibility of inoculation by human virus.
3. Immunization—Preventive vaccination after exposure to infection by inoculation.
4. Quarantine—None.
5. Concurrent disinfection of saliva of patient and articles soiled therewith.
6. Terminal disinfection—Thorough cleaning.

(B) General measures—

1. Muzzling of dogs when on public streets, or in places to which the public has access.
2. Detention and examination of dogs suspected of having rabies.
3. Immediate antirabic treatment of people bitten by dogs or by other animals suspected or known to have rabies, unless the animal is proved not to be rabid by subsequent observation or by microscopic examination of the brain and cord.
4. Annual immunization of dogs where the disease is prevalent.

SMALLPOX

1. *Infectious agent*.—Unknown.
2. *Source of infection*.—Lesions of the mucous membranes and skin of infected persons.
3. *Mode of transmission*.—By direct personal contact; by articles soiled with discharges from lesions. The virus may be present in all body discharges, including feces and urine. It may be carried by flies.
4. *Incubation period*.—Eight to sixteen days. (Cases with incubation period of twenty-one days are reported.)
5. *Period of communicability*.—From first symptoms to disappearance of all scabs and crusts.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms. Tests for immunity may prove useful.

2. Isolation—Hospital isolation in screened wards, free from vermin, until the period of infectivity is over.
3. Immunization—Vaccination.
4. Quarantine.—Isolation of all contacts until vaccinated with virus of full potency. Daily medical observation of all recently vaccinated contacts until height of reaction is passed, if vaccination was performed within twenty-four hours of first exposure, otherwise for sixteen days from last exposure.

SCARLET FEVER

1. *Infectious agent*.—*Streptococcus scarlatinae*.
2. *Source of infection*.—Discharges from the nose, throat, ears, abscesses or wound surfaces, and articles freshly soiled therewith. The nose and throat discharges of carriers may also spread the disease.
3. *Mode of transmission*.—Directly by personal contact with an infected person; indirectly by articles freshly soiled with discharges of an infected person, or through contaminated milk, or milk products.
4. *Incubation period*.—Two to seven days, usually three or four days.
5. *Period of communicability*.—Three weeks from the onset of the disease, without regard to the stage or extent of desquamation, and only after all abnormal discharges have ceased and all open sores or wounds have healed.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—By clinical symptoms.
2. Isolation—In home or hospital, maintained in each case until the end of the period of infectivity. If medical inspection is not available, isolation for twenty-eight days from onset.
3. Immunization—Exposed susceptibles as determined by the Dick test may be actively immunized by scarlet fever toxin.
4. Quarantine—Exclusion of exposed children and teachers from school, and food handlers from their work, until seven days have elapsed since last exposure to a recognized case.
5. Concurrent disinfection—Of all articles which have been in contact with a patient and all articles soiled with discharges of the patient.
6. Terminal disinfection—Thorough cleaning.

(B) General measures—

1. Daily examination of exposed children and of other possibly exposed persons for a week after last exposure.
2. Schools should not be closed where daily observation of the children by a physician or nurse can be provided for.
3. In school and institutional outbreaks immunization of all exposed children with scarlet fever toxin may be advisable.
4. Education as to special danger of exposing young children to those exhibiting acute catarrhal symptoms of any kind.
5. Pasteurization of milk supply.

SYPHILIS

1. *Infectious agent*.—*Treponema pallidum*.
2. *Source of infection*.—Discharges from the lesions of the skin and mucous membranes, and the blood of infected persons, and articles freshly soiled with such discharges or blood in which the *Treponema pallidum* is present.
3. *Mode of transmission*.—By direct personal contact with infected persons, and indirectly by contact with discharges from lesions or with the blood of such persons.
4. *Incubation period*.—About three weeks. (In rare instances reported to have been as long as seventy days.)
5. *Period of communicability*.—As long as the lesions are open upon the skin, or mucous membranes at any stage of the disease.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by microscopical examination of discharges and by serum reactions.
2. Isolation—Exclusion from sexual contact and from preparation or serving of food during the early and active period of the disease; otherwise none, unless the patient is unwilling to heed, or is incapable of observing, the precautions required by the medical advisor.
3. Immunization—None.
4. Quarantine—None.
5. Concurrent disinfection of discharges and of articles soiled therewith.
6. Terminal disinfection—None.

(B) General measures—

1. Education in matters of sexual hygiene, particularly as to the fact that continence in both sexes and at all ages is compatible with health and normal development.
2. Provision for accurate and early diagnosis and treatment, in hospitals and dispensaries, of infected persons, with consideration for privacy of record, and provision for following cases until cured.
3. Repression of prostitution by use of the police power and control of use of living premises.
4. Restriction of sale of alcoholic beverages.
5. Restriction of advertising of services or medicines for treatment of sex diseases, etc.
6. Abandonment of the use of common towels, cups, and toilet articles and eating utensils.
7. Exclusion of persons in the communicable stage of the disease from participation in the preparing and serving of food.
8. Personal prophylaxis should be advised to those who expose themselves to opportunity to infection.

TETANUS

1. *Infectious agent*.—Tetanus bacillus; *Clostridium tetani*.
2. *Source of infection*.—Animal manure, soil and street dust.
3. *Mode of transmission*.—Inoculation, or wound infection.

4. *Incubation period*.—Four days to three weeks, or longer if latent bacilli deposited in the tissues are stirred to activity by subsequent chemical or mechanical irritation. Commonly eight to ten days.
5. *Period of communicability*.—Patient not infectious except in rare instances where wound discharges are infectious.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms; may be confirmed bacteriologically.
2. Isolation—None.
3. Immunization—By at least one, and preferably two, injections of antitoxin.
4. Quarantine—None.
5. Concurrent disinfection—None.
6. Terminal disinfection—None.

(B) General measures—

1. Supervision of the practice of obstetrics.
2. Educational propaganda such as “safety-first” campaign, and “safe and sane Fourth of July” campaign.
3. Prophylactic use of tetanus antitoxin where wounds have been acquired in regions where the soil is known to be heavily contaminated, and in all cases where wounds are ragged or penetrating.
4. Removal of all foreign matter as early as possible from all wounds.
5. Supervision of biological products, especially vaccines and sera.

TUBERCULOSIS (PULMONARY)

1. *Infectious agent*.—Tubercle bacillus (human), *Mycobacterium tuberculosis (hominis)*.
2. *Source of infection*.—The specific organism present in the discharges, or articles freshly soiled with the discharges from any open tuberculous lesions, the most important discharge being sputum. Of less importance are discharges from the intestinal and genito-urinary tracts, or from lesions of the lymphatic glands, bone, and skin.
3. *Mode of transmission*.—Direct or indirect contact with an infected person by coughing, sneezing, or other droplet infection, kissing, common use of unsterilized food utensils, pipes, toys, drinking cups, etc., and possibly by contaminated flies and dust.
4. *Incubation period*.—Variable and dependent upon the type of the disease.
5. *Period of communicability*.—Exists as long as the specific organism is eliminated by the host. Commences when a lesion becomes an open one—i.e., discharging tubercle bacilli, and continues until it heals or death occurs.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—By thorough physical examination supplemented by use of the X-ray and specific skin reactions when necessary and confirmed by bacteriological examinations of sputum or other materials.

2. Isolation of such "open" cases as do not observe the precautions necessary to prevent the spread of the disease.
3. Immunization—None.
4. Quarantine—None.
5. Concurrent disinfection of sputum and articles soiled with it. Particular attention should be paid to prompt disposal or disinfection of sputum itself, of handkerchiefs, cloths, or paper soiled therewith, and of eating utensils used by the patient.
6. Terminal disinfection—Cleaning and renovation.

(B) General measures—

1. Education of the public in regard to the dangers of tuberculosis and the methods of control, with especial stress upon the danger of exposure and infection in early childhood.
2. Provision of dispensaries and visiting-nurse service for discovery of early cases and supervision of home cases.
3. Provision of hospitals for isolation of advanced cases, and sanatoria for the treatment of early cases.
4. Provision of open-air schools and preventoria for pretuberculous children.
5. Improvement of housing conditions and the nutrition of the poor.
6. Ventilation and elimination of dust in industrial establishments and places of public assembly.
7. Improvement of habits or personal hygiene and betterment of general living conditions.
8. Separation of babies from tuberculous mothers at birth.

TYPHOID FEVER

1. *Infectious agent*.—Typhoid bacillus, *Eberthella typhi*.
2. *Source of infection*.—Bowel discharges and urine of infected individuals. Healthy carriers are common.
3. *Mode of transmission*.—Conveyance of the specific organism by direct or indirect contact with a source of infection. Among indirect means of transmission are contaminated water, milk, and shellfish. Contaminated flies have been common means of transmission in epidemics.
4. *Incubation period*.—From seven to twenty-three days, averaging ten to fourteen days.
5. *Period of communicability*.—From the appearance of prodromal symptoms, throughout the illness and relapses during convalescence and until repeated bacteriological examinations of the discharges show persistent absence of the infecting organism.
6. *Methods of control*:

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, confirmed by specific agglutination test and bacteriological examination of blood, bowel discharges, or urine.
2. Isolation—In fly-proof room, preferably under hospital conditions, of such cases as cannot command adequate sanitary environment and nursing

care in their homes. Release from isolation should be determined by two successive negative cultures of stool and urine specimens collected not less than twenty-four hours apart.

3. Immunization—Of susceptibles in the family or household of the patient who have been exposed, or may be exposed during the course of the disease.
4. Quarantine—None.
5. Concurrent disinfection—Disinfection of all bowel and urinary discharges and articles soiled with them.
6. Terminal disinfection—Cleaning.

(B) General measures—

1. Protection and purification of public water supplies.
2. Pasteurization of public milk supplies.
3. Supervision of other food supplies, and of food handlers.
4. Prevention of fly breeding.
5. Sanitary disposal of human excreta.
6. Extension of immunization by vaccination as far as practicable in communities where the disease is prevalent.
7. Supervision of typhoid carriers and their exclusion from the handling of foods.
8. Systematic examination of fecal specimens from those who have been in contact with recognized cases, to detect carriers.
9. Persons who fail to show a strongly positive Widal reaction and contemplate traveling, should protect themselves by vaccination.
10. Exclusion of suspected milk supplies pending discovery of the person or other cause of contamination of the milk.
11. Exclusion of water supply, if contaminated, until adequately treated with hypochlorite or other efficient disinfectant, or unless all water used for toilet, cooking, and drinking purposes is boiled before use.

WHOOPIING COUGH

1. *Infectious agent*.—Pertussis bacillus of Bordet and Gengou, *Hemophilus pertussis*.
2. *Source of infection*.—Discharges from the laryngeal and bronchial mucous membranes of infected persons (rarely also of infected dogs and cats, which are known to be susceptible).
3. *Mode of transmission*.—Contact with an infected person or animal or with articles freshly soiled with the discharges of such person or animal.
4. *Incubation period*.—Commonly seven days, almost uniformly within ten days.
5. *Period of communicability*.—Particularly communicable in the early catarrhal stages before the characteristic whoop makes a clinical diagnosis possible. The catarrhal stage occupies from seven to fourteen days. After the characteristic whoop has appeared the communicable period continues certainly for three weeks. Even if the spasmodic cough with whoop persists longer than this it is most unlikely that the infecting organism can be isolated from the discharges. The communicable stage must be considered to extend from seven days after exposure to an infected individual to three weeks after the development of the characteristic whoop.

6. *Methods of control:*

(A) The infected individual and his environment—

1. Recognition of the disease—Clinical symptoms, supported by a differential leucocyte count, and confirmed where possible by bacteriological examination of bronchial secretions. A positive diagnosis may be made by bacteriological examination of laryngeal discharges as early as one week before the development of the characteristic whoop.
2. Isolation—Separation of the patient from susceptible children, and exclusion of the patient from school and public places for the period of presumed infectivity.
3. Immunization—Use of prophylactic vaccination recommended by some observers. Not effective in all cases.
4. Quarantine—Limited to the exclusion of nonimmune children from school and public gatherings for ten days after their last exposure to a recognized case.
5. Concurrent disinfection—Discharges from the nose and throat of the patient and articles soiled with such discharges.
6. Terminal disinfection—Cleaning of the premises used by the patient.

(B) General measures—

Education in habits of personal cleanliness and in the dangers of association or contact with those showing catarrhal symptoms with cough.

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CHAPTER XXVII

TRANSMISSION OF INFECTING AGENTS

The methods by which the agents which may cause disease are disseminated are quite varied. A number of these are worthy of discussion since they bear quite directly on human welfare and happiness. Some of our present-day ideas rest on explanations which have been handed down from former times before the days of experimental bacteriology. These are slowly being dispelled, however, and replaced by sounder explanations.

Longevity of Disease Bacteria Outside of the Host.—Whether an organism lives for a long time or for a short time in nature away from its host, influences to a great extent its significance as the cause of epidemics. The period of life depends upon a number of factors such as the characteristics of the organism in question, the intensity of the unfavorable factors, etc., but it is impossible to make general statements for all organisms. Data will be presented for only those organisms on which we have information founded on real experiment and which cause the more common infections. It will be readily appreciated that this is an important factor in determining whether any material is potentially dangerous in disease transmission or not. Many bacteria causing illness are unable to survive long away from living tissue. The longevity of the parasite largely determines the methods that are used for fighting it.

Eberthella typhi: (*Bacterium typhosum*).—Perhaps on account of the fact that typhoid fever is a disease of much epidemiological significance, the longevity of its etiologic factor, *Eberthella typhi* (*Bacterium typhosum*), has received considerable study. The conclusions reached by different investigators differ somewhat, as we might expect. During investigations incident to the famous controversy in 1901 between the City of St. Louis and the State of Missouri and the City of Chicago and the State of Illinois, this question was thoroughly studied by both sides. St. Louis contended that pouring of Chicago sewage into the Illinois river endangered the sanitary quality of her drinking water. It was argued that disease bacteria might come down the Illinois river. Much experimental work was necessary before this argument could be refuted or confirmed. As far as pathogenic bacteria were concerned most of the work centered

about *Eberthella typhi* (*Bacterium typhosum*). The data from these experiments seemed to indicate that the organism could survive in pure water (Lake Michigan water) for about eight days and in impure water (Chicago Drainage Canal) for four days. Former experiments had shown a survival period of as long as two months in sterilized water and in unsterilized water up to several weeks. We may then take an algebraic sum of the evidence and reach the conclusion that *Eberthella typhi* (*Bacterium typhosum*) may persist in natural waters for upwards of several weeks.

Infection by Carriers.—A carrier is a person who harbors pathogenic microorganisms in or on his body without showing symptoms of infection. As in the missed case, the carrier is a menace to the health of the community. One of the worst features of the situation is that the carrier shows no symptoms and often does not know that he is a menace to those about him. The reports of carriers are especially interesting for it required epidemiological investigations just as careful and logical as criminal investigations in many cases, to find out the real source of the objectionable bacteria. The relation of carriers to food infection has been discussed in Chapter XXV.

From reports in the literature, a person may apparently remain a carrier for a long time after he has recovered from the disease. One report deals with a case of a man who had typhoid fever fifty-four years before; another with a case who had the same disease thirty years before. Such data and others which could be mentioned, seem to suggest that a person may remain a carrier his whole life long after infection.

Some carriers cannot be cured while others may be cleared up. When they cannot be cured and are unwilling to submit to well-established practices for their control, then the government is justified in taking away their liberties. That is done in other situations. If a person refuses to obey the law of society which states that he shall not kill a fellow human being, he is set apart from the rest of society in prison, if not put to death. Society makes its laws for the greatest good of the greatest number.

The Missed Case.—This is one of the most insidious problems with which preventive medical efforts have to cope. The missed case, or unrecognized case, like the carrier may be responsible for certain endemic diseases such as diphtheria, common colds, etc. As medical information increases, the missed case seems to become more significant. The distinction between a missed case and a carrier is perhaps not always apparent. Usually in the missed case there are a few symptoms although they may not be severe enough to cause concern. Individuals differ greatly in their reaction to symptoms of illness. Some yield and

secure the advice of physicians who diagnose their illnesses; others resist and either go about their work until they finally have to yield, or recover. If the latter have suffered from a communicable disease they may have been responsible for other cases.

INFECTION BY FOMITES

A fomite is an inanimate agent which spreads material causing disease. The term is usually applied to communicable diseases. The fomite has probably been greatly overemphasized as an agent in the spread of disease. One prominent health officer has stated that, if fomites were potent factors in the cause of disease, most of us would be ill most of the time. A few so-called fomites will be discussed.

Books.—The relation of books to the spread of disease is another example of the possible significance of fomites in public health. The problem is a serious one for libraries and school authorities. It is possible that our information does not rest on sufficient data. It might be profitable to report briefly one or two investigations on this subject. Laubach studied the question in three different ways: (1) an investigation of 75 soiled and torn library books that for several years had been passing through the hands of children who had been living under very undesirable sanitary conditions; (2) a search for diphtheria bacilli on 150 books which were known to have been handled by persons ill with diphtheria, and (3) a study of books artificially contaminated with *Escherichia coli*, *Eberthella typhi* and *Corynebacterium diphtheriae*. The 75 library books showed only the presence of *Escherichia coli*. It was stated that since *Eberthella typhi* did not differ much from *Escherichia coli*, the latter organism might exist equally long. The examination of the 150 books for diphtheria bacilli yielded negative results. Artificial inoculation of books showed that *Escherichia coli*, *Eberthella typhi* and *Corynebacterium diphtheriae* remained alive for months, and also on the outsides of books which were kept in the dark. It is difficult and perhaps wrong to attempt specific conclusions from these experiments. From the data secured by artificial inoculation of books it would seem best not to use books which had been directly exposed to infectious material until after disinfection. It is interesting to note that sunlight was efficient for this purpose. Kenwood and Dover have stated that there is probably no material risk involved in the reissue of books recently read by consumptives unless the books are obviously soiled and even then the risks are very slight.

Money.—Recent experimental work seems to indicate that much misinformation is held on this subject. On account of the fact that

money is rapidly circulated between many individuals, one might expect it to disseminate pathogenic bacteria. If this were true, we might have a difficult problem with which to cope. Fortunately, coins carry very few bacteria. It is known that the metals from which the coins are made allow the formation of salts which are toxic to microorganisms. A penny is made up of 95 per cent of copper and 5 per cent of silver and tin; a nickel 75 per cent of copper and 25 per cent of nickel and a dime 90 per cent of silver and 10 per cent of copper. The salts of all of these metals, with the possible exception of tin, are known to be bactericidal. As a coin is passed on in circulation, it might come in contact with acids and alkalis which would cause salt formation. Even paper bills contain few bacteria due, perhaps, to the fact that the conditions for survival on them are not favorable to the bacteria.

Postage Stamps.—On account of the construction of a postage stamp, it might easily pick up objectionable bacteria. This idea is illustrated in the instructions to postmasters that they shall dispense stamps with the gummed side up to minimize the opportunity for acquiring bacteria from the counter. One study of stamps from fifty different sources showed ordinary bacteria among which were several forms that may, at times, be pathogenic. The danger, however, from postage stamps is very slight since they are not handed about, ordinarily, as are coins. One cannot overlook, however, the practice of moistening the stamp with the tongue. A recent report of a laboratory examination of the bacterial content of postage stamps reveals numerous bacteria. In some cases the colonies were too numerous to count.

Dishes and Tableware.—Such fomites come into very close contact with the sick and thus may harbor active disease-producing bacteria. Taylor showed the presence of living tubercle bacilli on eating utensils used by persons ill with tuberculosis. Hemolytic streptococci were isolated from dishes and tableware in restaurants by another investigator. From these data and others which could be given, one will appreciate readily the danger of infection that lurks in the utensils for dispensing beverages at public places. In America the public drinking cup has been eliminated by legislation in practically all places except the so-called soft-drink parlor. Here the superficial methods of washing are a menace and one may be convinced that he is using common eating utensils unless paper utensils are used.

Bacteriology of Dishwashing.—This procedure in the home may be an important one especially if a communicable disease is present. Since the great epidemic of respiratory infections in 1918-1919, considerable work has been done. These studies revealed the great danger that exists unless utensils are intelligently washed. The experimental

work has shown that machine-washed dishes are much more satisfactory bacteriologically than are hand-washed dishes. Hand-washed dishes are washed, usually, at a much lower temperature. With machine-washed dishes boiling water may be used. Oram and Broadhurst found that the hottest water which thirty different people could use (49–57° C.) was not germicidal since it was below even pasteurizing temperatures and acted for too short a time. They also reported that hot water alone did not remove or destroy throat organisms. Hand-washed dishes, it was advised, should be rinsed in boiling water. A soapy lather was necessary to entirely remove material with which the dishes were contaminated before the washing experiments. The day will come in America when more attention will be paid by public health officials to this phase of public health activity. Glass containers which are only superficially washed will not be tolerated in public drinking places.

Washing Powders.—Such powders are frequently used in water for washing dishes and dairy utensils. Doane studied the germicidal properties of some common washing powders. He noted a destruction of bacteria by most of those which were used. He stated, however, that the choice of a washing powder would rest on the price and not a superior germicidal activity of any one powder. The discussion of the germicidal activity of soaps given in Chapter IX also has some bearing here.

It is proper to point out at this place that Schroeder and Southerland, who studied the bactericidal effect of soaps in laundering, found them of no value in the strengths used. Their data are indirectly applicable to this discussion. They were able to isolate a staphylococcus from a soap solution which was ten times as strong as the solution used in the washing machine. Such data indicate that, if disinfection is desired, a detergent should be used first followed by the disinfectant.

Bathroom Appliances.—These might be regarded without reservation as suspicious fomites. Some data have been published in our literature from which conclusions may be reached. Hirst and Rosenberg studied over 100 tubs in three college dormitories. About 35 per cent of the tubs showed the presence of *Escherichia coli*, which we have learned is an indicator of pollution. These investigators reported that the indicator organism was more frequently found on the bottoms of the tubs than on the sides. Especially important, however, was the statement that visual criteria of cleanliness were without value in predicting the hygienic condition of the tubs. This paper is of especial interest in connection with a report by Dowd in 1921 of a gonorrheal infection traced to a bath tub. The author was absolutely convinced of the correctness of his assumption.

Soiled Linens.—The relation of such fomites to disease is so close that little evidence need be presented. It is easy to appreciate the possibility that such things as bed linen, sleeping garments, and the like, might be dangerous. For instance, McIntosh, a British medical officer of health, reached the conclusion after a painstaking investigation that soiled linen from a home in which smallpox existed had caused the infection of laundry workers. Several investigations have been carried out by public health workers to ascertain the effects of laundering procedures on bacterial life. Such an investigation has been reported by Schroeder and Southerland.¹ Their study involved two types of laundries, hand laundries and steam laundries. In general, they found the hand laundries to be unsatisfactory. The methods of sorting and marking the clothes might lead to infection of the employees. They found that in most hand laundries the clothes were placed in large bags or nets and sent to steam laundries where they were washed as units and returned to the hand laundries wet. The drying facilities in hand laundries were not such that the death of objectionable bacteria would be secured, if they survived the washing process. In the case of steam laundries there was greater opportunity for satisfactory results but certain objectionable practices were observed. Soiled and clean clothes were carried in the same wagon. Wet clothes infected with bacteria and subjected to the action of the usual degree of heat found in drying houses, tumblers, mangles and hot presses, were freed of living bacteria. Such reports, and others that might be mentioned, justify consideration of the public laundry as a possible danger. To give a better idea of the methods used by Schroeder and Southerland, the following paragraphs are quoted.

To prove the efficiency of the washing processes when restricted as to time and temperature, but not limited in regard to soaps, bleaches, or water, a series of laboratory tests was arranged. The testing materials consisted of strips of half woolen and woolen goods, 6 by 9 inches. These strips were dipped into water containing *Bacillus coli* and approximating 8,120,000 bacteria per cubic centimeter. The prepared tests used in the earlier experiments were employed also and filled with *coli* broth which contained approximately 812,000,000 per cubic centimeter. The inoculated material and the tubes were wrapped in towels, tied, and placed among the clothes which were to be passed through the washing processes.

The machine was filled with small nets, weighing approximately 2 pounds each. The washing was then done as follows: two cold rinses, five minutes

¹ Schroeder, M. C., and Southerland, S. G. Laundries and the Public Health. A Sanitary Study Including Bacteriologic Tests. Public Health Reports, **32** (1917), 225-246.

each; one hot suds, ten minutes duration; total amount of time, twenty minutes.

The results of these experiments showed comparatively slight reduction of the bacteria in either the prepared tests or the broth tubes in the watery suspension.

A second series of experiments was worked out with an increased time limit (fifty-four minutes for total processes and a temperature limit of 93.5° F.). This, as we had been assured by the laundrymen, represented the average temperature plus a slightly increased time limit employed for colored clothes and flannels. No bactericidal destruction took place.

A third series of experiments with the same time limit but at higher temperatures was also tried. With nets averaging 25 pounds each and a temperature of 150° F. there was a reduction of 50 per cent in the number of bacteria present in the tests inclosed in the center of the nets.

These experiments show conclusively that the temperature and time limits employed were wholly inadequate to destroy bacteria of the pathogenic type.

We may also refer to a recent study at the University of Nebraska on the bacterial content of undershirts which are worn frequently without washing. From an average bacterial content of 400,000 per square inch after one use the number increased to nearly 10,000,000 after the garment was worn six times. Laundering reduced the number and with drying caused a satisfactory elimination of bacteria. The bacteria present were found to be *Staphylococcus albus* and *Staphylococcus aureus*. Hemolytic types were common. (Jour. Home Economics, May 1928.)

Attention is also directed to the discussion in Chapter X of ironing as a means of sterilization.

Other Fomites.—With a little thought other fomites come to mind. Saelhof reported that hemolytic streptococci and occasionally *Corynebacterium diphtheriae* and pneumococcus were present on the receivers and transmitters of telephones. So-called court plaster may be considered as a fomite. It is prepared long before it is used and even though it is prepared from sterile materials, it may become objectionable during its sojourn in the home before use. It is quite unsatisfactory for wounds. It is frequently moistened with saliva before application to wounds which makes it still more objectionable.

AIR-BORNE INFECTION

At one time air was thought to play an important rôle in the spread of disease. One of the theories of disease was founded in part on the presence of emanations or some hypothetical agent in the air. Perhaps there was some reason for such an attitude. Our forefathers observed that many who lived in the vicinity of swamps had malaria. Consequently the disease was called swamp-fever and it was attributed to

emanations or gases given off from the swamp. Further experimental work indicated that the agents given off by swamps were animate in the form of mosquitoes.

The location of hospitals and tuberculosis sanitariums is often beset with much opposition on the part of citizens who reside near the proposed sites. They believe that they stand a greater chance of contracting disease. Eminent students of public health have stated that there is no basis for the belief that those living about a hospital are more liable to have communicable disease. Isolation hospitals, previously termed pest-houses, were usually located away from the hamlets in order to reduce the danger believed to exist. Such was the inconvenience and hardship caused by misinformation. Rosenau has stated that the more transmission of communicable disease is studied, the less air is implicated.

Much concern was caused our forefathers by the belief that sewer air was especially dangerous. This was often called sewer gas. Considerable experimental data were manipulated to give experimental foundation to this theory, but later work showed that another explanation had to be given. To-day, we believe that sewer air or sewer gas is not poisonous and probably does not contain more undesirable bacteria than many other types of air. This does not indicate that one should go out of his way to breathe sewer gas.

CONTACT INFECTION

Contact infection, meaning the actual contact of diseased or infected surfaces with healthy surfaces, is the most direct method for disseminating the virus of disease. There is little need for such explanations as indirect contact for explaining the spread of disease. Such means are better taken care of by other expressions. Contact infection explains certain observations better than the original explanations. The committee which prepared the report on the "Control of Communicable Diseases" defined the term contact as follows: *a "contact" is any person or animal known to have been sufficiently near an infected person or animal to have been presumably exposed to transfer infectious material directly, or by articles freshly soiled with such material.*

While almost any disease may be spread by immediate contact, there are some which certainly fall into this category better than others. Such are syphilis and gonorrhea. They are the best examples of contact diseases. While there is a little evidence that they may be contracted from intermediate agents, the possibility is remote. They are generally

disseminated by immediate contact of diseased individuals with well individuals.

The topics discussed in a text-book are quite liable to be more or less abstract. This is always the case unless personal experience has taught us the facts. In order to show that contact is a method by which disease may be spread, there is quoted below an account of a smallpox peddler.² It is possible that the reading of such an account will help to emphasize more satisfactorily the significance of *contact* in disease dissemination than a long discussion.

About October 15, 1921, a man arrived in Garnett, Anderson County, from Kansas City, Mo., to work with a road gang whose camp was immediately outside Garnett. He was in the early stage of black smallpox, and two or three days later reported sick. The disease was promptly recognized, the man isolated, and all of the other men in the gang vaccinated. The problem then arose as to who was to attend this man. Working with the gang was a man named W—— who professed to have no fear whatever of smallpox, and to whom the task of nursing a sick man appeared easier than building roads. He therefore offered his services to the county health officer, and it was agreed that he should act as nurse for this man and keep himself rigidly isolated from the outside world.

While the nursing apparently suited W——'s taste, the solitude and isolation certainly did not, and he frequently took the liberty of going to town for one reason or another. Being warned by his companions that he was breaking the law and fearing that he should be put under arrest, he boarded a train one night and disappeared.

Nothing was heard of him until two weeks later, when he appeared in Iola, Allen County, and reported sick. His sickness proved to be smallpox and this was promptly recognized. A vacant house was procured and the man confined there. In the meantime the man in Garnett died, but W——, more fortunate than his former patient, after ten days or so of fairly severe illness felt practically well as ever—so well, in fact, that he decided to leave Iola and get married.

The wedding trip included a visit to Thayer, Neosho County, and in this place he called on three families. This was about November 18. During the first week of December smallpox broke out in these families, and at time of writing fourteen cases of smallpox have occurred, all of which can be directly or indirectly traced to W——.

During the first week of December also the bride became ill, and later on her sister and mother-in-law (Mrs. W——, Sr.). It does not seem to have occurred to W——, however, that all these cases could have had anything to do with his sickness, and while his wife was sick, and later his mother, he

² Pedley, Frank G. A Smallpox Peddler. Kansas State Board of Health, Bul. 17, pp. 222-223 (1921).

kept constantly leaving the house where they were confined and associating with well people.

From the fact that his operations involved three counties, the association of this man with all these cases was not at first noticed, but when it became apparent he was immediately placed under arrest and is now awaiting his trial.

From this man's ignorance and criminal carelessness have apparently arisen at least fourteen cases of smallpox and four deaths—one of them his own mother. The mayor of Thayer, writing to the State Board of Health, stated that some of the cases there were so horribly disfigured by the disease that they "did not look like human beings."

This history is given not only for its interest, but to emphasize the fact that people of the type of W—— are to be found everywhere. In fact, at times we are completely at their mercy, and the only way to be perfectly safe is to be vaccinated.

The evil of handshaking has recently been discussed by Hill and Mathews. This is another illustration of close contact which may result in dissemination of undesirable bacteria. The danger which lurks in the practice is borne out by the many cases of typhoid fever caused by carriers who prepared the food. In such cases, the hands have probably been the agents by which the foods were infected. The hands which infected the foods may have infected other hands. A little thought will soon convince the most skeptical that the hands may be dangerous agents. All sorts of things and materials are handled from morning until night. Handshaking allows the most intimate contact and the reasons for which it was introduced by our early forefathers no longer exist in most communities. Buice and his colleagues recently reported the occurrence of *Escherichia coli* of intestinal origin on the hands of one out of every twelve food handlers examined. There is no reason to believe that these observations might not be extended to other groups of people. During the War when influenza was raging through practically all nations, its dissemination was said to be by the hands in some cases.

INSECT-BORNE DISEASES

Such diseases as yellow fever, malaria, and Rocky Mountain spotted fever are spread by insects. Besides these, in which the microorganism seems to develop in the body of the insect, are other diseases such as typhoid fever and dysentery in which the microorganisms are carried on the insect. The fly, mosquito, cockroach, and bedbug all more or less common insects, have been studied bacteriologically. We may spend most of our time in analyzing the case against the fly. During the Spanish-American War, a great many American soldiers had typhoid

fever. A commission appointed to investigate the question decided that flies served as the carriers of the typhoid bacteria.

Reports on the bacteriology of flies are numerous in the literature. In one case an investigator examined flies for typhoid bacteria near a sick room, in which was a typhoid patient; five out of eighteen flies showed the presence of this organism. Esten and Mason reported that a single fly could carry from 550 to 6,600,000 bacteria of all kinds. It is unnecessary to quote more data. The case against the fly is well established.

The fly is of interest as an agent for the spread of disease-producing bacteria in two ways: first, the mechanical carrying of microorganisms on the feet and legs, and secondly in the alimentary tract. The latter method may need a little more discussion than the former. Graham-Smith, who made a careful study of the subject, fed flies on a number of pathogenic bacteria and then determined the longest period after which organisms could be recovered from various parts of the fly's body. The data were interesting. They appear in Table IX. Graham-Smith called attention to the fact that these data came from heavily infected flies.

TABLE IX

SHOWING THE LONGEST PERIOD AFTER WHICH PATHOGENIC BACTERIA COULD BE ISOLATED FROM ARTIFICIALLY INFECTED FLIES
(AFTER GRAHAM-SMITH)

Time in days

	Legs	Wings	Head	Crop	Gut	Feces
Eberthella typhi.....					6	2
enteritidis.....	7		7	8	7	
Mycobact. tuberculosis..				3	16	13
Tuberculous sputum.....					7	5
Yeast.....	2.5	2.5	2.5	2	3	2
Corynebacterium diphtheriae.....	5	5	5	7	5	2
Bacillus anthracis.....	2		4	5	3	2
cholerae.....	30 hrs.	5 hrs.	2	2	2	30 hrs.
Serratia marcescens.....	8	12	11	5	17	6
Anthrax spores.....	20	20	20	13	20	13

The cockroach, *Blatta germanica*, has also been incriminated in the dissemination of undesirable bacteria. One investigator stated that the cockroach, by contamination with its feces, is able to play a part in the

dissemination of tuberculosis and the pyogenic bacteria. Animal and vegetable parasites have been demonstrated in the digestive tubes of cockroaches. A South American worker found pathogenic bacteria in cockroaches caught in toilet rooms. Barber, in 1914, also showed that the cockroach should be stamped out. He exposed fecal material which had been inoculated with the organism causing Asiatic cholera; the roaches devoured this greedily. The bacteria were demonstrated in the roaches from six to nine hours after feeding. The cockroach might infect food during this time since the cholera parasite will live for about sixteen hours on human food after discharge by the roach. Such data indicate a strong case against the cockroach.

The red ant was shown to harbor the cholera organism eight hours after it had ingested food containing it.

Working with fleas, Bacot showed that the alimentary tract of the flea larva may become infected with bacteria if these bacteria appear in the food. He also showed that infection of the larval gut might persist until the resting period of the larva in the cocoon and that there was satisfactory evidence that such infection might survive the pupal stage.

Under this heading we have an opportunity to discuss some of the early work which was done to prove the fact that yellow fever was a mosquito-borne disease. As is true with many of our great discoveries, indefinite statements were made in early medical work that yellow fever was spread by mosquitoes. It remained for some carefully controlled experiments by Reed, Agramonté, Lazear and Carroll to prove this definitely beyond doubt. By means of experimental work in Cuba these medical officers of the United States Army secured experimental proof for the hypothesis expressed by Findlay in 1881. The experiments were outlined in a careful manner and yielded logical conclusions. The experiments involved the use of voluntary human beings who yielded themselves to the bites of infected mosquitoes. First of all, of course, these men had to be carefully isolated for a period greater than the incubation period of yellow fever to insure that they were healthy individuals with which to conduct the experiments. Having passed this test the men were placed in screened enclosures in which infected mosquitoes were released. Yellow fever usually resulted in about five days. Other experiments were conducted which confirmed the conclusion that the yellow fever mosquito was the real agent in the transmission of the disease.

Experiments were also conducted to prove that other agents, which were at one time or other believed to disseminate the disease, did not transmit it. For instance, it was believed at one time that bed linen and articles of clothing could harbor the infectious matter and thus be

responsible for the dissemination of the disease. This was disproven by allowing healthy men to sleep in beds soiled with excreta from yellow-fever patients. Such experiments were negative. These and other experiments too numerous to mention helped to strengthen the case against the mosquito. Having proven that the mosquito spread the disease, anti-mosquito campaigns were instituted in certain tropical cities. These in themselves caused a great reduction in the yellow fever case rate as well as the mortality. The conquering of yellow fever is one of the greatest triumphs of sanitary science. It has made possible the building of the Panama Canal, a project which was several times defeated by mosquitoes. It has made life and happiness more certain in tropical cities.

ANIMAL-BORNE DISEASES

These diseases include some of the most interesting and important ones with which we have to deal. Some of them are discussed at other places in this book. Several of the important ones will be mentioned.

Anthrax.—This disease has been characterized in Chapter XVI. It is a very malignant disease of sheep and cattle and may be contracted by eating the flesh of animals which have died of the disease, or through abrasions in the skin through which the parasite enters. In this last relation, cheap shaving brushes have been especially significant. These were finally placed under governmental control so that sterilization could be enforced. The disease is also an industrial disease since those engaged in the handling of hides have shown a high incidence of infection.

Glanders.—Glanders is a disease of horses which may be communicated to man. It was described as follows by the committee which prepared the report on the Control of Communicable Diseases.

GLANDERS

1. *Infectious agent.*—Glanders bacillus, *Pfefferella mallei*.
2. *Source of infection.*—Discharges from open lesions of mucous membranes, or of the skin of human or equine cases of the disease (i.e., pus and mucus from the nose, throat, and bowel discharges from infected man and horse).
3. *Mode of transmission.*—Contact with a case or with articles freshly soiled by discharges from a human or equine case.
4. *Incubation period.*—Unknown.
5. *Period of communicability.*—Until bacilli disappear from discharges or until lesions have healed.
6. *Methods of control:*

(A) The infected individual and his environment—

1. Recognition of the disease—By specific biological reactions, such as the complement fixation test, the mallein test, the agglutination test, or by

nonspecific reactions, such as the Straus reaction, if confirmed by culture, or by identification of the *Pfefferella mallei*, or by autopsy of doubtful cases.

2. Isolation.—Human case at home or hospital; for infected horses destruction rather than isolation is advised. **Skin contact with the lesions in the living or dead body is to be scrupulously avoided.**
3. Immunization.—None of established value or generally accepted.
4. Quarantine of all horses in an infected stable until all have been tested by specific reactions, and the removal of infected horses and terminal disinfection of stable have been accomplished.
5. Concurrent disinfection—Discharges from human cases and articles soiled therewith.
6. Terminal disinfection—Stables and contents where infected horses are found.

(B) General measures—

1. The abolition of the common drinking trough for horses.
2. Sanitary supervision of stables and blacksmith shops.
3. Semiannual testing of all horses by a specific reaction where the disease is common.
4. Testing of all horses offered for sale where the disease is common.

NOTE.—In this disease, as in all infectious or communicable diseases from which both animals and humans suffer, cases occurring in animals should be reported to the Department of Agriculture and human cases should be reported to the Department of Health, reciprocal notification thereafter to be accomplished through official interdepartment channels.

Tuberculosis.—This disease is usually thought of first of all when one thinks of diseases which are transmissible from animals to man. The discussion has centered around the possibility of transmitting bovine tuberculosis to human beings. In 1898, Smith distinguished the bovine type of tubercle bacillus³ from the human type. In 1913, Park and Krumwiede gave some figures which probably represent the present-day situation. All pulmonary tuberculosis is caused by the human type of the bacillus; about one-tenth of the tuberculosis of bones, joints and lymph nodes in adults and a fourth of tuberculosis of this type in children are due to the bovine bacillus. Other data have indicated that from 6 to 10 per cent of deaths from tuberculosis in children below five years of age are due to the bovine type of tubercle bacillus. This is probably due to the fact that milk constitutes a larger part of the food of children than of adults.

FOOD-BORNE INFECTIONS

This subject has been discussed in several other places. Such infections differ only slightly from those caused in other ways. In this case

³ There are four types of tubercle bacilli according to most medical investigators, human, bovine, avian and fish. The first two interest us here. The names of these types according to some of the more recent classifications are given in the chapter on Classification of Bacteria.

the causal microorganism is disseminated by some food. The foods may become infected either through handling by a carrier or by being naturally infected during production.

METHODS FOR PREVENTING SPREAD OF DISEASE

All of the methods for combating and stamping out disease may be separated into two groups. These may be considered as subjective methods and objective methods. Both types should be used. A person should not trust his safety and freedom from disease to either group of methods alone. One should supplement the other.

Subjective Methods.—These are preventive measures applied to the patient, or subject of infection, and consist of the general procedures by which a person may build up his resistance but especially of the methods of preventive inoculation and immune reactions. These are discussed in later chapters.

Objective Methods.—Many of the objective methods are discussed at some length in other paragraphs. These are the methods which may be applied to factors in one's environment such as water disinfection, milk pasteurization, disinfection procedures, quarantine, etc.

QUARANTINE METHODS

These methods are perhaps the most important of the objective methods that may be applied for preventing the spread of disease bacteria. They involve the isolation of the infected individual and thus decrease his significance as a focus of infection. Quarantine procedures rest on the same social principles that cause us to jail certain members of society and to place others suffering from mental ill health in hospitals. It is for the greatest good of the greatest number.

An early quarantine act was enforced in 1403 by Venice; a small island was used as a quarantine station and incoming vessels were required to remain there for forty days. From this (Fr. *quarantaine*) arose our modern term.

International or Maritime Quarantine.—An international quarantine is rigidly enforced by the United States of America to prevent the entrance of cases of certain diseases. Six diseases are so dreaded by sanitarians that they are watched for in international quarantine work; they are *smallpox*, *leprosy*, *yellow fever*, *typhus fever*, *cholera* and *plague*. Persons attempting to enter this country on a vessel from a country where these diseases are epidemic are carefully examined. If a case of one of the above diseases appears on board a vessel, that vessel with its crew and passengers is held at a quarantine station until the period of

incubation for the disease is exceeded. This gives an opportunity for any who have been exposed to the disease to become ill. The quarantine procedures are well known but it is well to point out that to-day a quarantine officer may know in a short time the incidence of disease in a foreign country. Formerly he had to depend on documents brought by each vessel to this country. Those especially interested in this subject should seek information on the United States Bill of Health.

The infectious diseases not included in the above list are not so greatly feared and consequently cases are allowed to enter this country. They become subject to the laws of the state in which the port of entry is located.

Federal or Interstate Quarantine.—This type of quarantine can be enforced only by federal officers of health. It has been employed for the control of foot-and-mouth disease in cattle, the interstate shipment of tuberculous cattle, etc. While it is not strictly a matter of quarantine, we may point out that federal bureaus control the water supplied on interstate carriers, the condemnation of unfit food seized in interstate traffic, etc.

State Quarantine.—This is enforced by state laws and is usually administered by the State Board or Department of Public Health. Such state bureaus have jurisdiction only within states and although there is usually perfect harmony, they are not subject to the federal health bureaus.

Municipal Quarantine.—This is enforced by city officers and is usually founded on the state quarantine laws. It is the type of quarantine with which the layman is most familiar. It has been defined as follows by a committee of eminent health authorities:

Quarantine: By quarantine is meant the limitation of freedom or movement of persons or animals who have been exposed to communicable disease for a period of time equal to the longest usual incubation period of the disease to which they have been exposed.

Detention Camp.—The detention camp is used in times of military emergency, for instance, to prevent the introduction of diseases into large groups of men. If troops are moved from an area where disease is prevalent to an area where it is not, they may be placed in a detention camp for a period longer than the incubation period of the disease which is of interest at the time.

Sanitary Cordon.—A sanitary cordon is a form of quarantine or isolation which is enforced in times of emergency.

Isolation of the Patient.—This is the most direct way of segregating the infected person. Could the sick be absolutely isolated until after

such time as they can excrete pathogenic bacteria, disease could be more easily controlled. This term was defined by a committee of the American Public Health Association as follows:

By isolation⁴ is meant the separating of persons suffering from a communicable disease, or carriers of the infecting organism, from other persons, in such places and under such conditions as will prevent the direct or indirect conveyance of the infectious agent to susceptible persons.

Isolation of the patient is probably one of the best procedures for stamping out infections. If rigidly enforced there would be no opportunity for pathogenic bacteria to be disseminated. However, it seems too difficult to isolate a patient absolutely, for there are some infections, such as influenza, in which isolation in the ordinary sense of the term has not accomplished much in their control.

What has been said about isolation of the patient seems to be true also for communities and groups of people. Dr. H. K. Marshall made the following statement about the experiences of certain of the Fiji Islands during the pandemic of influenza in 1918.

During this epidemic I was a member of the British medical service of the colony of Fiji. Some instances of the effectiveness of isolation against infection of communities with influenza came under my notice at that time which I think might be of interest, as they confirm the conclusion that "it is quite safe to assert that perfect isolation of an individual or group during an influenza epidemic constitutes a complete protection against the disease."

An instance of the effectiveness of isolation by means of quarantine measures occurred in American Samoa, which was under the command of an American naval officer. The port of entry here is Pago Pago. Efficient quarantine prevented infection of the inhabitants of American Samoa with influenza. Pago Pago is approximately 60 miles distant from Apia, which is the port of that part of the Samoan group administered by the government of New Zealand. Thus, while British Samoa was ravaged with influenza, American Samoa did not suffer from the disease.

The little island of Rotumah is located about 200 miles north of the main Fiji group. This island escaped infection during the epidemic for the reason that it was entirely cut off from communication with the outside world. Ordinarily, a schedule of monthly communication between Suva, Fiji and Rotumah is maintained. During the occurrence of influenza in Suva, this schedule was interrupted and no boat was dispatched to Rotumah for a period of three months. It was then found that this island had escaped the infection.

⁴ In view of the various ambiguous and inaccurate uses to which the words isolation and quarantine are not infrequently put, it has seemed best to adopt arbitrarily the word isolation as describing the limitation put upon the movements of the known sick or "carrier" individual or animal, and the word quarantine as describing the limitations put upon exposed or "contact" individuals.

A resourceful planter who lived and owned a large plantation at a place called Taviuni on Vanua Leva, Fiji, kept his district free of infection by means of an efficient quarantine. Although districts around him were infected heavily, the area which he isolated remained free from infection until long after the peak of the incidence of infection of the epidemic had been reached. The cases that occurred subsequently were of only mild character.

The Makogai Leper Asylum of Fiji also escaped the ravages of the epidemic. I can speak from personal knowledge of the facts concerning this instance, as at that time I was the acting medical superintendent of the asylum. The asylum is located on a small island separated from Levuka, the nearest port by 18 miles. At that time it had a population of about 400 persons; 350 patients with leprosy and 50 personnel. Until August, 1919, no cases of influenza occurred. By virtue of the quarantine restrictions which the government had in force regarding the island of Makogai at all times, an efficient quarantine against influenza was carried out easily. It was necessary to make trips to Levuka in order to obtain supplies, but it was possible during these trips to avoid close contacts. Levuka suffered heavily from the infection.

How Disease Bacteria Leave the Body of the Patient.—In order for a sick person to communicate disease to another, the microorganisms causing his illness must leave his own person in some way. In some cases they may be carried by insects and other agents which have been discussed elsewhere. In this place, we will mention the methods by which bacteria may leave the body.

From the Mouth: The mouth with its saliva makes a fertile soil for many different bacteria. There are several bacteria which cause disease in the mouth. If such diseases exist, the bacteria may be easily excreted in discharges from the mouth. The spitting habit is not only disgusting but equally dangerous. This has been recognized for a long time in the case of tuberculosis. Persons afflicted with that disease are instructed very early in the care of their sputum.

Coughing, sneezing or even ordinary speaking cause many bacteria to be expelled from the mouth. Winslow found that speaking caused bacteria to be thrown out of the mouth for a considerable distance.

In the Stools.—The excretum from the alimentary tract is especially important in certain diseases such as typhoid fever, dysentery, etc. This may reach a water course supplying untreated water to a community or it may reach food by means of soiled hands to give rise to a carrier-borne outbreak of disease such as those discussed in a former chapter. When epidemiological investigations suggest a carrier as the cause of an undue amount of disease in a locality, the search starts with examination of feces from those who are handling food. Besides the diseases in the group mentioned above, there are other diseases the eti-

ologic agents of which reach the feces somewhat indirectly. Thus tuberculosis bacteria may reach the feces by being swallowed in sputum.

In the Urine.—Certain pathogenic bacteria such as *Eberthella typhi* may be excreted in the urine. In the search for typhoid carriers both excreta and urine should be examined. In some cases, the failure to examine the urine has caused confusion in the solution of carrier-caused typhoid fever epidemics. Tubercle bacilli may also be excreted in the urine. The bacteriological examination of urine is often made inaccurate by faulty methods for collecting the sample. Sterile catheters should be used and every attempt made to prevent the ingress of extraneous bacteria.

In the Nasal Excretion.—The nasal excretion would, of course, be significant in the respiratory diseases.

The Milk.—Research has indicated without doubt that the milk of animals may contain pathogenic bacteria. From published reports we may conclude that the human mother may also excrete pathogenic bacteria in her milk. In one report typhoid fever bacteria appeared in the milk of a woman on the eleventh day of the disease. The nursing, aged twenty-five days, died of typhoid fever two weeks later. In another case, colon bacilli were found in the milk of a woman who suffered from sudden fever and digestive disturbances. An extensive study of the bacteriology of human milk was conducted on 100 women in London. Forty-nine per cent of these specimens showed streptococci, only 2 per cent of which indicated pathological conditions in the breast. In several cases *Escherichia coli* was isolated. Normal human milk, it was said, might contain *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococci* and *Escherichia coli*. Such data indicate that human milk, like cows', is not sterile when excreted but may contain bacteria. Human milk, therefore, may be responsible for infections in infants.

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CHAPTER XXVIII

FACTORS INFLUENCING INFECTION

Our former discussions have shown that disease depends upon many different factors and upon the intensity of these factors. Some of them are of less significance than others and play a sort of indirect rôle but still have some influence. Those which are more apparent will be tabulated below with brief discussion. Undoubtedly many others could be discussed but in a book of this nature this is unnecessary.

Heredity.—It is not surprising that many believe infectious disease to be inherited. The spread of disease in a family would, at first thought, lend support to the belief. However, such disease is now best explained by contact. The members of a family have many opportunities for contact infection. Some of the disagreements in opinions over the influence of heredity on disease is perhaps due to different definitions of some of the terms. Heredity probably has something to do with susceptibility and resistance to disease. Davenport¹ has explained it on the same basis as other inheritable characteristics. He stated, "It thus appears that in inheritance of our traits and, among others, in inheritance of those somatic conditions which permit the development of disease, we are to a large extent what our chromosomes make us."

Age.—Some diseases also show an age incidence. Such diseases as mumps, chicken-pox, and measles are more commonly diseases of childhood; others such as heart disease and cancer are diseases of old age. Sharp lines of demarcation cannot be made when considering characteristics and functions of living organisms and they cannot be made here. It is known, for instance, that older people infrequently have some of the diseases of childhood.

Mental State.—This is another remote, but often important, factor to be considered among those which influence disease. It probably does not influence greatly the course of an infection unless it prompts one to neglect it; death may follow under such conditions more quickly. Psychic therapy probably has a place in present-day medicine, but is to

¹ Davenport, C. B., Heredity and Disease. Jour. Amer. Med. Assn., **87** (1926), 664-667.

be distinguished from many other terms and practices that may be confused with it.

Occupation.—Diseases which are directly due to trades or professions are spoken of as occupational diseases. They are often so serious that many states have bureaus empowered by law to study them. Such diseases are lead poisoning, certain types of pneumonia, furunculosis due to contaminated cutting oils, etc. Such diseases are also spoken of as vocational diseases.

Housing Conditions.—This is perhaps more indefinite as a factor influencing infection. The resistance to disease which an individual possesses is also lowered by overcrowding in homes. Crowded living conditions cause closer contact which gives the best conditions for spreading infectious material. The influence of living conditions has received much study in tuberculosis, a disease which has reaped vengeance on homes in the crowded tenements. While the tenements in large cities are easily connected with tuberculosis and kindred diseases, it is not so easy to admit that housing conditions in rural sections may also have significance. Yet such is the case, if the account reported by Palmer² indicates the situation. This is a terrible tale of tuberculosis in a house in the outskirts of a rural community in Illinois. Perhaps the story has been overdrawn but there is enough foundation to it to stimulate our cooperation in all efforts to reduce and eliminate disease.

Temperature.—The temperature of the environment may also indirectly influence disease incidence and immunity reactions. Dr. Victor Vaughan, for many years dean of medicine at the University of Michigan, in his interesting volume, "A Doctor's Memoirs," stated his belief that the high temperatures of the middle western states in summer and fall greatly increased the incidence of "bloody dysentery," a very common infection forty or fifty years ago. Winslow and his colleagues also found that temperatures of 84° F. to 89° F. caused a distinct decrease in the rate of hemolysin formation on the part of animals. If these observations with hemolysin represent the situation for the other immune bodies, we have a more rational explanation for the detrimental effects of higher temperatures.

Closely related to temperature is the seasonal incidence of disease. Respiratory infections are more common in the late fall and winter. Typhoid fever is a later summer-fall infection.

Fatigue.—The relation of fatigue to disease has become more and more apparent as investigations have been carried out. This factor in

² Palmer, Geo. T. The Story of a Country House. Illinois Health News, 1 (1915), 69-71.

disease was recently discussed as follows in the *Journal of the American Medical Association* (Vol. 86 (1926), p. 753).

Although fatigue is a phenomenon that every person recognizes readily in a subjective way, physiologists still are struggling with the objective interpretation of its nature. One may read of the alleged accumulation of "fatigue substances," notably in the muscles, and of the circulation of products of overwork that are not ordinarily present in the blood; but a well-founded theory of the nature of fatigue remains to be formulated. It is known that prolonged muscular activity, and particularly contractile effort under the influence of a lessened supply of oxygen, may lead to appearance of unusual quantities of lactic acid, among other demonstrable substances. These can exert noticeable changes on various bodily functions, such as respiration, the heart beat, and the irritability of the muscle itself. The mere statement of such demonstrable effects, however, gives only partial insight into the far-reaching consequences that may be involved.

One of the "antidotes" to fatigue is rest, which affords an opportunity for recuperation. There is a widespread belief that resistance to infection is promoted by bodily rest, and a common method of combating it consists in promoting rest. A recent writer has even referred to the doctrine that the pain of injuries is beneficial because it secures local rest, which promotes healing; and he suggests that it may reasonably be extended to the general malaise of infections. It might be assumed as a possible corollary of this that fatigue conduces to susceptibility to infection. Experiments to test this hypothesis have not been entirely lacking. Abbott and Gildersleeve,³ observing that animals fatigued by running exhibit a depression of the opsonic index, concluded that they must have a greater susceptibility to bacterial infection. The experiments of Spaeth at Johns Hopkins University lent no support to such a conclusion. On the other hand, observations of Boycott and Price-Jones at the University College Hospital Medical School, London, reopen the entire question. They demonstrate that under certain circumstances fatigue does promote infection. All-inclusive statements should not be made. Thus, an amount of exercise sufficiently severe to delay substantially the normal increase of weight of growing animals did not break down their natural resistance to tuberculosis. The feature deserving of emphasis at this time is the necessity of more exact information on a large variety of specific features of possible interrelations between fatigue and disease. At a period when industrial efficiency and the bodily welfare of the worker is a prominent topic of serious discussion, the bearing of the possible relation of fatigue on the causation and the prevention of infectious diseases in industrial work is sufficiently obvious. Lee⁴ has, indeed, pointed out that the identification and treatment of industrial diseases and the appreciation of industrial

³ Abbott and Gildersleeve. *The Influence of Muscular Fatigue and of Alcohol on Certain of the Normal Defenses*, Univ. Pennsylvania M. Bull. 23, 169, 1910.

⁴ Lee, F. S. *The Human Machine*, New York, Longmans, Green & Co., 1918. *Fatigue and Resistance to Disease*, editorial, J. A. M. A. 79, 2165 (Dec. 23), 1922.

hazards to health and ways of preventing them are parts of the general modern recognition of the importance of the individual and the duties of society toward him. Here, Lee adds, the physician, the philanthropist and the legislator have worked in helpful partnership.

Plant pathologists have also reported observations which bear on this discussion. They have found that if plants which are weakened, are sprayed with spores of an organism which is pathogenic for them, they will be more quickly infected; normal healthy plants sprayed at the same time will not succumb but will remain healthy.

IMMEDIATE FACTORS INFLUENCING INFECTION

After discussing the remote factors influencing infection, there are several immediate factors for consideration. It must have been appreciated by this time that infection is a complicated phenomenon depending not only on a great number of factors but also on their intensity.

The Subject of Infection.—Two factors are necessary for a case of communicable disease, the subject of infection and the infecting agent. The outcome of the infection depends, therefore, on the resistance of the infected and the virulence of the microorganisms. We may now enumerate some of the specific factors involved. This allows us to appreciate the fact that disease is the result of a conflict between two forces, the resisting powers of the host and the assaulting power of the parasite.

Resistance of Patient.—This is important since a person's resistance may be lowered by careless habits of living or raised by proper habits. It is an indefinite entity, however, to discuss. Search has been made for satisfactory methods of estimating resistance but none have been discovered which have enjoyed wide acceptance. Some experiments with animals have been carried out, all of which have some bearing on this discussion. It has been found that dogs become more susceptible to disease when fatigued and hungry. This may explain the greater incidence of disease among troops in the field, especially those who are living under bad conditions with inadequate rest and food.

Avitaminosis.—The lack of the necessary so-called accessory substances, vitamins, in the diet has also been more recently shown to be a predisposing factor in disease and illness. In general, it may be stated that vitamin-deficient diets so reduce the vitality of an individual that he may become more susceptible to the attacks of bacteria. Werkman, using rats, pigeons, rabbits and bacteria causing disease affecting these animals, was able to observe a consistently lowered resistance among the animals on a deficient diet than among the controls on a normal diet.

In one case, six rats suffering from lack of vitamin A and six normal control rats were injected intraperitoneally with *Bacillus anthracis*. All of the test rats died while the control animals all lived. Cramer and his colleagues also published evidence supporting the above statements. They noticed a marked decrease in blood platelets among rats fed on a vitamin A deficient diet. They believed that resistance to infection was related in some manner to the platelet content of the blood. While one may not wish to accept all of the statements in publications on this subject there is sufficient evidence to regard avitaminosis as an important predisposing factor in infection. A more recent investigation has suggested a close relationship between production of rickets and susceptibility to tuberculosis. The white rat is very susceptible to rickets and quite resistant to tuberculosis. Consequently, Grant fed young rats on rations adequate with the exception of calcium and the anti-rachitic factor. Rickets appeared more readily in cloudy weather than in bright weather. Such animals injected with *Mycobacterium tuberculosis* were easily infected. These experiments seemed to have been sufficiently controlled and indicate that an apparently simple dietary deficiency may lower the resistance to infections. Rickets is a common disease and such experiments give it added significance.

EXTERNAL DEFENCES

Under this heading may be mentioned several different factors which may be regarded as external defences.

The Skin.—The skin may be considered as an impassable barrier to infecting organisms which may lodge on it. As long as it is not broken these organisms cannot enter. It is not difficult to isolate from the surface of the body *Streptococci* which are pathogenic when introduced into the blood. In the mouth are frequently found such pathogenic bacteria as *Meningococci* and *Pneumococci*. Such information gives more support to the laws of personal hygiene which attempt to stimulate body cleanliness

Furunculoses (Boils, Pimples, Carbuncles, etc.).—These are common infections of the skin quite often looked upon as minor matters; they possess, however, sufficient possibility to cause serious results and should be looked upon with some concern. The theories by which infections of boils have been explained are old. One of the explanations held even to-day by some is that boils are due to poisons in the blood. The boil was looked upon as an attempt on nature's part to get rid of this poison. In order to assist nature, a poultice was applied which, it was said, would draw out the poison. Such a practice often resulted in the appearance

of several other boils about the original one. This was desired and caused no worry. It was said that the poultice was drawing out more poison and that consequently more boils were necessary. At the same time that such a treatment was applied the patient was given medicine to "purify" the blood.

A sounder, more rational explanation is now accepted for the cause of boils. To-day, they are recognized as due to an infection. The pathogenic bacteria lodge on the skin and, if an abrasion is made, penetrate to establish a focus which we speak of as a boil. Such information indicates that better methods for treating boils should be used than the older methods described above. If boils are due to infections great care should be used to prevent the bacteria from the first boil from infecting adjacent portions of the skin to cause more foci of infection. Poultices are not conducive to this since they cause the matter extruded from a boil to be spread about adjacent portions of the skin. Boils should be opened by a physician and a safe disinfectant applied about the boil to destroy bacteria. The appearance of boils one after another or the spreading of one boil over a restricted area indicates the lack of adequate disinfection.

External Defences of the Alimentary Tract.—The alimentary tract has secretions which may exert a detrimental action on some pathogenic microorganisms. The *saliva* has been studied in this respect and while one investigator could observe a decrease in the number of cells exposed to its action, the saliva cannot be looked upon as a potent factor in disease prevention.

The *gastric juice* in the stomach has received more study. Some writers state that the gastric juice is a very active germicide. Others very wisely make more cautious statements. Some of the early work on this subject was carried out by Spallanzani, who secured gastric juice by swallowing small sponges with strings attached. After the sponges had been in the stomach long enough to have soaked up some gastric juice, they were drawn out and the juice expressed. When this gastric juice was rubbed over meat, the meat did not undergo rapid putrefaction. Many years later when they were studying cholera, Pettenkofer and Emmerich carried out an experiment which is often quoted in this connection. They drank some water to which living cholera microorganisms had been added. One added sodium bicarbonate which neutralized the gastric juice allowing the cholera organisms to pass through the stomach unharmed. A more severe case of cholera resulted.

While there are data to show that the gastric juice is germicidal, there are also observations which seem to refute it. The causal organisms of several of the common diseases are known to be taken in either

food and drink and therefore pass through the stomach since the lesions are in the intestines. They may escape the action of the gastric juice by passing through the stomach imbedded in food particles or in water which not only dilutes the gastric juice but passes through the stomach quickly.

The *bile* is also germicidal for some bacteria. This is borne out in reports of many investigators who have studied the selective action of the bile on bacteria. It has been known for some time that immediately below the point where the bile is poured into the intestines, there is a pure culture of *Escherichia coli* (*Bacterium coli*). This would indicate that the bile had an antagonistic action on other bacteria but either no inhibitory action or an accelerating effect on *Escherichia coli*.

The Infecting Agent.—The course of the infection depends greatly on the characteristics of the parasite or infecting agent. The sum total of them give the bacterium its aggressive power to combat the host.

Virulence or Infectiosity.—Just as with the factor of “resistance of the host” this is a difficult factor to measure. The virulence of a bacterium is measured by inoculating some susceptible animal or plant. The grade of virulence, then, is really subject to the grade of resistance which the experimental animal has. Like many other characteristics, virulence is not permanent but varies and, in reality, is a very unsatisfactory characteristic to evaluate. Many data show that microorganisms vary greatly in virulence. This characteristic is subject to many conditions under which the organism is cultured. It may be purposely reduced or raised by certain methods of culture. Cultures of some pathogenic bacteria become rapidly devitalized by continued propagation in the laboratory. Such an organism is *Bacillus anthracis*. Animal passage is often used for strengthening the activity of an organism. The variation in virulence has caused the introduction of polyvalent bacterins discussed later.

Number of Cells.—This also is another factor upon which it is difficult to secure specific experimental data. “How many cells are necessary for disease causation?” Unfortunately, this cannot be easily answered. Here again, the resistance of the host has to be considered. It is quite conceivable that for one host one cell might cause the disease while for another 100 cells might not. Probably every individual is able to tolerate a certain number of cells of average virulence; a heavy inoculation or massive dose would, however, cause the disease despite resistance or immunity even though it be raised by inoculation procedures. Thus, to a certain extent, disease depends on the same factors as poisoning.

Avenue of Infection.—Much depends on this factor. Some micro-

organisms are significant only when introduced by one avenue. Others cause severe results by one avenue but very light infections by other avenues. This may be nicely demonstrated in the laboratory with *Staphylococcus aureus*. When a virulent strain is injected into the blood stream of a rabbit, a fatal septicemia ensues; however, a subcutaneous injection usually causes only a local infection which suppurates and finally heals. Another illustration that is often mentioned is infection with *Clostridium tetani*. Probably the spores of this microorganism are frequently eaten; they cause no harm in the intestinal tract, but let these spores get into the blood stream, a terrible toxemia results.

The Alimentary Tract.—Many of the materials taken into the alimentary tract are not sterile. When such foods and drinks contain harmful bacteria, and when these are able to pass through the stomach, disease may result. That the alimentary tract is an important avenue of infection is indicated by the prevalence of intestinal infections. The causal organism is taken in with the food.

The Lungs.—Infected dust particles may produce infections in the lungs. The best-known lung infections are pneumonia and tuberculosis. Nature has provided agents in the respiratory tract to help protect the lungs from infection but these at times may be inadequate.

The Skin.—The skin is an impassable covering when it has no abrasions through which bacteria may enter underlying tissue or the blood stream. These abrasions do not have to be large; even the smallest may allow the entrance of bacteria causing serious infections. These organisms may also reach the hair follicles and establish a focus of infection.

The mucous membrane which lines the urinary, alimentary, and respiratory tracts is also said to possess germicidal properties which prevent infection. If such a property exists, it may be due either to a germicidal action of the mucus or to a mechanical wearing away of this membrane. However, there are a number of observations which suggest that the protective action of the mucosa may be over-emphasized. Certain bacteria must be able to penetrate it and cause disease. Gonorrhea probably results from the penetration of the mucosa by the *Gonococcus*. There are numerous reports in medical literature indicating that the virus of smallpox may also penetrate the mucosa. Individuals have been accidentally vaccinated in the eyes, nostrils, etc.

CHAPTER XXIX

MODES OF BACTERIAL ACTION

Pure and Single Infections.—Single infections are caused by but one microorganism or parasite. Such infections are often local in character. Lobar pneumonia is a good example of such an infection. This is probably due to the fact that the lesion or infected focus is located in the bronchioles of the lungs; other bacteria cannot readily enter. Infection of the urinary tract by *Escherichia coli* (*Bacterium coli*) is usually a pure infection. Other infections that are pure infections are cerebrospinal meningitis, anthrax and furunculosis (boils). Typhoid fever may also be a pure or single infection.

Mixed Infections.—A mixed infection is one in which two or more parasites are concerned. Such infections are much more common on the exposed surfaces of the body where various organisms may enter. Much confusion has occurred in the study of some diseases by the investigators reporting one of the contaminating microorganisms to be the etiologic agent. Many infections which are pure, single infections in some stages, are mixed infections in other stages. Very often the entrance of a pathogenic microorganism may stimulate the transformation of bacteria normally present into parasites; this complicates the situation as far as treatment is concerned.

There is much evidence that one organism not necessarily very pathogenic may pave the way for a second one. Large typhoid fever epidemics are often preceded by a large number of diarrheal cases. Kendall has stated that mild catarrhal inflammations often precede epidemics of cerebrospinal meningitis. It is also known that outbreaks of diarrhoea frequently precede typhoid epidemics.

Having introduced the subject of bacteria which are always present becoming harmful, it may be profitable to discuss them further. Kendall grouped bacteria which incite human infections into two groups, sporadic infectants or "opportunists" and those which cause disease progressive from man to man. The former, the opportunists, live normally on mucous membranes, in channels or cavities opening freely to the surface of the body, for they may be regarded as surface growers. They are unable to penetrate to underlying tissue and have to wait for

injury. Then, they may cause severe infections but ordinarily do not cause epidemics.

The second group, few in number, includes those bacteria decidedly inimical to man. They are the tissue growers and are the typical pathogenic forms. Unlike the "opportunists" they do not have to wait for injury but are aggressive enough in themselves to gain entrance.

MODES OF BACTERIAL ACTION

One often hears the question, "How do bacteria cause disease?" A general answer cannot be given this question. It depends on the organism, the avenue of entrance, the experimental animal or patient, and other factors.

Muir and Ritchie in their "Manual of Bacteriology" have answered the question by stating that there are two main factors involved: (a) the multiplication of living organisms after they have entered the body and (b) the production by them of poisons which may act on the tissues. The former phenomena have been given some consideration in another place and the latter, involved in the phenomenon of intoxication, will be discussed below.

Conditions Affecting Pathogenicity.—The ability to cause disease seems not to be limited to a few bacteria. Some of the commonest saprophytes such as *Bacillus subtilis* have been repeatedly isolated from infections where they seemed to have played the most important rôle. There is scarcely an organism which has not, at some time or other, been connected with pathologic conditions. The specific factors which determine pathogenicity have really been discussed in Chapter XXXVIII. They, of course, are inherent in the organism; but pathogenicity, as we have learned, is determined to a great extent by the characteristics of the infected animal.

Among the various changes or effects which bacteria bring about when they produce disease, two may be mentioned.

Tissue Changes.—Some bacteria produce very characteristic tissue changes while others do not. Some lesions are characterized by a sloughing away as is the case in leprosy and to a far less extent in boils. In such cases the infected organism tries to defend itself, thus giving rise to an inflamed area. Most diseases have some characteristic lesion where the etiologic agent is especially located.

Intoxication.—Intoxication may be defined as a poisoned state of the body; it is synonymous with toxemia. Many different substances may cause intoxication. Some of the better-known intoxications are caused by ethyl alcohol, strychnine, mercuric chloride, etc. To these may now

be added the bacterial intoxications which result in such diseases as diphtheria, tetanus, botulism, and others. It is well known that in all cases of poisoning an antidote is given. The antidotes (antitoxins) for bacterial poisonings are discussed in Chapter XXX. Such antidotes are not chemical compounds but substances which have been found to bring about the same results that known antidotes do in other poisonings. In general, the symptoms of intoxication are somewhat alike. Comparison of the symptoms of botulism, tetanus, toadstool poisoning, etc., show many in common at least during the earlier stages of these diseases. Of course they differ at times, which allows the physician to distinguish them.

BACTERIAL TOXINS

Toxins are poisons formed by living cells. They are not limited to bacterial cells, for the production of poisonous substances is a widespread characteristic among many forms of life. The student will find it profitable to review the subject of enzymes since much of the information on enzymes may be applied to toxins.

Not much is known about the purpose of toxin in the life of the organism which forms it. Different explanations may be offered. Toxins may be products of excretion which happen to be poisonous to other forms. They may be formed by the cell as protective agents or they may be the result of the action of the microorganism on the medium.

Toxin Formation by Other Species.—Several different species of animals and plants form toxins, poisonous substances. Spiders¹ are known to cause serious cases of illness after biting. *Lathrodectus mactans* has been said to be the most poisonous species in America. Certain jelly fishes are venomous. A Coelenterate known as "Portuguese man-o-war" (*Physalia*) causes pronounced symptoms of illness following the sting. Snakes are, perhaps, the best-known animals that produce poisons although much of our knowledge does not rest on sound observations. The rattlesnake is a poisonous American species. The vipers are also known to be poisonous. Snake venoms are not unlike bacterial toxins. They stimulate the production of antitoxins when injected into an animal and the antitoxins (antitoxic serum) may be used for the treatment of other cases of snake bite.

Plants also form poisons. The poisonous constituent of toadstools has been studied by Ford who reported some properties like those of bacterial toxins. The castor bean has been shown to contain a virulent poison, ricin.

¹ Bogen, E. Arachnidism, A Study of Spider Poisoning. Amer. Med. Assn., 86 (1926), 1894-1896.

Kinds of Toxins.—When the enzymes were discussed, it convenient to separate them into two groups depending on whether they acted inside of the cell or outside of the cell. The same separation may be made for the toxins giving a group known as *extra-cellular* (*exotoxins*) or *soluble toxins* and another group known as *intracellular* or *insoluble toxins* (*endotoxins*). This distinction is very useful because it helps us to understand the relation of different bacteria to disease, differences between the methods of active and passive immunity, etc.

The extracellular or soluble toxins are those which diffuse through the cell walls and appear in the medium. They are, then, somewhat more comparable to snake venoms than the intracellular toxins (endotoxins). Once these toxins are excreted from the cell, they are free and have no more to do with it.

Bacteria producing soluble or exotoxins with the diseases produced are:

Name	Disease Produced
<i>Clostridium botulinum</i>	Botulism
<i>Clostridium tetani</i>	Tetanus (lockjaw)
<i>Corynebacterium diphtheriae</i>	Diphtheria
<i>Streptococcus scarlatinae</i>	Scarlet fever
<i>Clostridium welchi</i>	Gas gangrene

The intracellular toxins or endotoxins are firmly bound in the cell. They are not excreted through the cell wall. Consequently, in order to secure such toxins, the cells must be ground up and the suspension filtered through a sterile porous filter. The toxin appears in the filtrate in which there should be no living cells.

Bacteria forming insoluble or endotoxins with the diseases produced are as follows:

Name	Disease produced
<i>Eberthella typhi</i>	Typhoid fever
<i>Salmonella paratyphi</i>	Paratyphoid fever
<i>Microspira cholerae</i>	Cholera
<i>Pasteurella pestis</i>	Plague
<i>Bacterium dysenteriae</i>	Dysentery (Bacillary)

Characteristics of Toxins and Toxin Reactions.—The characteristics of toxins are not unlike those of enzymes. They will be reviewed here in outline form.

1. *Toxins are formed by living cells.* Toxins have never been synthesized by biochemists. They are made by living cells. Could we know the chemical constitution of toxins, it might soon follow that we

would be able to understand the antitoxins and arrive at a place where chemical compounds could be used for neutralizing toxins in the blood.

2. *Toxins and toxin reactions are specific.* Each toxin has special affinity for certain body parts. Their specificity is, perhaps, their best-known characteristic and is especially apparent in their reactions with antitoxins. There is a special affinity between diphtheria toxin and diphtheria antitoxin. The antitoxin neutralizes or inactivates the toxin. However, diphtheria antitoxin has no affinity for tetanus toxin. This specificity is useful in the treatment of disease and also in the identification of unknown bacteria which are toxin formers. The toxin formed in syphilis shows its effect in two ways. It may attack the afferent conducting portions of the spinal cord to produce locomotor ataxia, or it may attack certain portions of the brain to cause paralysis and insanity. The toxin formed by *Clostridium tetani* shows special attraction for certain neurones.

3. *Toxins are active in minute quantities.* This characteristic is exceptionally interesting. Before one can secure information on this question, it is necessary to control as many variable factors as possible. This is often difficult to do. However, enough data are available to indicate that toxins are active in very, very small amounts. The data are also greatly influenced by the method of administration of the toxin as well as the size of the animal. The two toxins which have received most study in this respect are those of *Clostridium tetani* and *Clostridium botulinum*. Van Ermengem, who isolated the latter of these organisms, calculated, on the basis of the dose fatal for a rabbit, that one-thirtieth mm. of dried toxin would be fatal to a 70-kg. man, if injected subcutaneously. "Brieger and Cohn estimated that if 0.000,000,05 gram of their strongest tetanus toxin was fatal to a mouse weighing 15 grams, the fatal dose for a 70-kg. man would be 0.000,23 gram, or about one-fourth of a milligram."² Miss Bengtson worked with the toxin of *Clostridium botulinum* and stated that the strongest toxin which was produced was for the type B strain, the fatal dose of which for a 250-gram guinea pig was 0.000,000,03 gram. On this basis, she computed the fatal dose for a 70-kg. man to be 0.000,008,4 gram. As she pointed out, the toxin of *Clostridium botulinum* seems to be more poisonous than the toxin of *Clostridium tetani*.

4. *Toxin reactions are reversible.* When toxin unites with antitoxin, a compound is formed which, while stable under ordinary circumstances, is capable of breaking up under certain conditions.

5. *Toxins stimulate formation of antitoxins.* This characteristic has

² Quoted from Bengtson.

received extended discussion later on. It is a very important characteristic. The fact that each toxin is able to stimulate the formation of specific antitoxin is used as the basis for certain practices in therapeutics.

6. *They possess certain properties of living cells.* Like enzymes, toxins have some of the properties of living cells. Toxins are thermostable—destroyed by high temperatures.

7. *In developing their effects a period of incubation is required.* A certain period of time seems to be required before the cells in the body of the host are sufficiently poisoned to show symptoms of illness. This depends largely on the strength or potency of the toxin as well as on the amount which the person receives.

8. *Toxin reactions in the host usually set up characteristic symptoms.* Fever is one of the most common; there are many of these, such as general malaise and wasting away.

Constituents of Toxin.—The term toxin is a general one which includes all of the untoward properties of toxic filtrates. As information has increased, this agent has been divided into several parts. For instance, Ehrlich announced two constituents in toxin formed by *Clostridium tetani*. One he called *tetanospasmin*—the portion which acted on the nerves yielding the symptoms for which tetanus is known; the other *tetanolysin* which destroyed the red blood corpuscles. *Tetanospasmin* was easily destroyed by heat while *tetanolysin* was more resistant. Not all bacterial toxins have been broken up into such constituents. The toxin formed by toadstools has been found to contain two distinct constituents. The existence of these constituents in toxin might, at least, explain some of the variations in symptoms.

Structure of Toxins.—Unfortunately, very little is known about the structure of toxins. They cannot be identified chemically. In order to make them and their reactions more easily understood Ehrlich used graphic illustrations. He depicted the toxin molecule as a body with two functioning parts, a haptophore group and a toxophore group. The haptophore group served to bind or anchor the toxin molecule to a body cell after which the toxophore portion functioned to poison the cell. This illustration has done much toward making the understanding of the action of toxins more intelligible. For instance, it is known that some

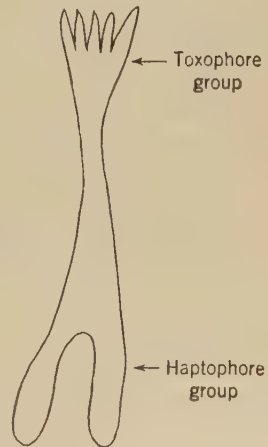


FIG. 128.—Showing a Toxin Molecule According to the Conceptions of Ehrlich.

toxin preparations lose their ability to poison cells but still retain their ability to unite with or bind antitoxin. This means that the toxin molecule has been altered in such a manner as to destroy its toxophore group while the haptophore groups has remained intact. Such a toxin molecule is called a *toxoid*. Ehrlich's explanation has some ardent critics. We need not be concerned with these controversies but use the theory if it helps to make the situation more clear.

Toxins vs. Ptomaines.—Toxins and ptomaines are often confused in students' minds. If we admit for the moment that ptomaines are poisonous they may be distinguished from bacterial toxins in several ways. Ptomaines are cleavage products of the materials upon which the bacterium grows. Bacterial toxins, on the other hand, are produced probably by internal processes of the bacterial cell. Bacterial toxins are heat labile while the ptomaines are probably not.

CHAPTER XXX

PROTECTIVE SUBSTANCES—IMMUNE BODIES

ANTIBODIES

As soon as it was known that a person was immune or resistant to certain diseases after having the disease or receiving preventive inoculation, an explanation for the observations was sought. A number of protective agents, called immune bodies or antibodies, are now known, which are used for explaining these phenomena. None of them has been separated in the pure state, but their existence can be proved by delicate tests and by what they accomplish. The situation, then, is not greatly different from that which exists with enzymes, filterable virus, etc.

Antigens and Antibodies.—These are two convenient terms used by bacteriologists; they are collective terms and permit a great saving of time. An antigen is a substance which, when introduced into an animal, causes the formation of *immune bodies* or *antibodies*. An *antibody* or *immune body* is an agent formed in the blood of an animal which has received an antigen. The antigen may be either animal or vegetable products, toxin, bacteria, enzymes, etc. In order to secure the formation of antibodies, one must inject into a susceptible animal an antigen which will cause this animal to form them. The formation of antibodies or immune bodies is a protective act on the part of the animal. It should be mentioned also that if animal products are used as antigens, an animal of unrelated species must be used, else no antibodies will be formed. The following are some of the more common antibody reactions:

Antibodies or Immune Bodies		Antigen
1. Antitoxins	+ Toxin	= Inactivation of Toxin
2. Agglutinins	+ Bacterial cells	= Agglutination
3. Precipitins	+ Proteins	= Precipitate
4. Opsonins	+ Bacterial cells	= Opsonized bacterial cells
5. Lysins		
Bacteriolysins	+ Bacterial cells	= Lysis
Cytolysins	+ Cells	= Cytolysis
Hemolysins	+ Red blood corpuscles	= Hemolysis (laking)

One of the most important characteristics of both antigens and antibodies is that they are specific. Antigens are specific because each antigen is capable of stimulating the formation of an antibody which is reactive with itself and no other antigen. Therefore, antibodies are specific; this makes them useful for treating disease. Diphtheria antitoxin, for instance, is useful only for the destruction of diphtheria toxin. It has no effect on tetanus toxin or other toxins.

Terms Used.—Much confusion in terms exists for the agents used in preventive inoculation. A *serum* is a material prepared from the blood of some animal. When it contains antibodies or immune bodies, it is spoken of as an *antiserum*, or *immune serum*. When no immune bodies are present it is called normal serum. Two types of antisera are known, *antitoxic sera* and *antibacterial sera*, depending on the antigen which was used for preparing them. For the production of antitoxic sera, toxins are used as the antigens. If tetanus toxin is used the serum is spoken of as antitetanic serum; another such antitoxic serum is antidiphtheritic serum. With some bacteria as has been shown above, we are unable to prepare the toxin free from the bacterial cells. To prepare an antibacterial serum the cells themselves have to be used as antigens. These are the antibacterial sera such as antigonococcic serum, antistreptococcic serum, antimeningitic serum, etc. Other terms often confused with those just discussed are vaccine and bacterin. They are discussed later on in this chapter. The student should think his way through with these definitions for they can be understood only in this manner.

ANTITOXINS (ANTITOXIC SERA)

Antitoxins are immune bodies which neutralize or counteract the action of toxins. Consequently, they may be used for prevention and cure of diseases caused by toxins which are excreted from the cells. This is not a new idea. Antidotes are always given to counteract poisons. When a person suffers from arsenical poisoning he is given an antidote; when he suffers from diphtheria an antidote is also given. In the latter case, however, resort must be made to an antitoxin or antitoxic serum, for chemical compounds as antidotes in bacterial poisoning are unknown. Antitoxins may be used prophylactically or therapeutically. When used in the former manner they are used to prevent possible toxemia; in the latter case, they are used as curative agents after the poisoning has started.

Preparation of Antitoxic Sera.—Such agents for preventive inoculation must be made in the blood stream of some animal. As has been discussed in an early chapter in this book, Emil von Behring is given

credit for the discovery of antitoxins, antidotes for bacterial poisonings. The preparation of antitoxins (antitoxic sera) will be outlined and discussed.

Production of Toxin.—In order to cause the system of some animal, such as the horse, to form protective substances (immune bodies, or antibodies) against diphtheria toxin, an antigen, diphtheria toxin, must be used. This toxin is made by growing the diphtheria bacilli in a veal broth medium in which the cells find the proper food substances. During growth, they pour out of their cells a potent toxin which accumulates in the medium about the cells. During incubation the bacteria grow in a thin pellicle on the surface of the medium. As time continues the pellicle thickens and part of it falls to the bottom of the culture flask. At the end of the incubation period, usually about a week, the medium is sterilized by treatment with an antiseptic and by passing it through a sterile porous filter such as has been described in an earlier chapter. This yields a filtrate containing the toxin from which the cells have been removed. Manufacturers give much attention to the strains of micro-organisms used for this purpose. They are kept active by frequent animal passage and regular subculture. Then, only those cultures are used which can produce a large amount of antitoxin in the body of a horse. In America a strain of *Corynebacterium diphtheriae*, known as Parks' No. 8, is used because it forms an especially active poison or toxin.

Animal Used.—For quite obvious reasons the horse is used for antibody formation. The chicken and goat are also useful. It would take about as much time to immunize a chicken or goat as a horse. From the horse a much larger yield of serum is secured. The animals to be used in antitoxin work are carefully selected and kept under observations for a few months to insure freedom from disease. Some horses seem to form little antitoxin. Some cases have been reported where toxin injections for as long as two years have failed to stimulate antitoxin production in the blood. After the horse is shown to be free from disease by qualified veterinarians, it enters the antitoxin laboratory where it is given the immunizing treatment against the toxin of the organism causing the disease for which the antitoxic serum is being prepared.

Immunizing the Horse.—After the strength of the toxin has been determined as mentioned above, it is given to the horse in slowly increasing amounts. It is necessary to start with small doses since a poison is being introduced which, if injected at first in too large doses, would cause the death of the horse. The injections are made under the skin and in this way the absorption is slower. The first dose, which may be

about 0.5 cc. in size, causes a rise in temperature and the usual other symptoms of illness such as a rough coat, etc. Another dose, larger than the first, is given in twenty-four hours. This process is repeated until the animal can tolerate enormous doses. Toward the end of the immunization period the horses may receive doses of toxin as large as 1000 cc. During these injections, nature is striving to counteract this poison, by the formation of an antidote, which may be called an antitoxin. One paper reported that the maximum dose of toxin injected

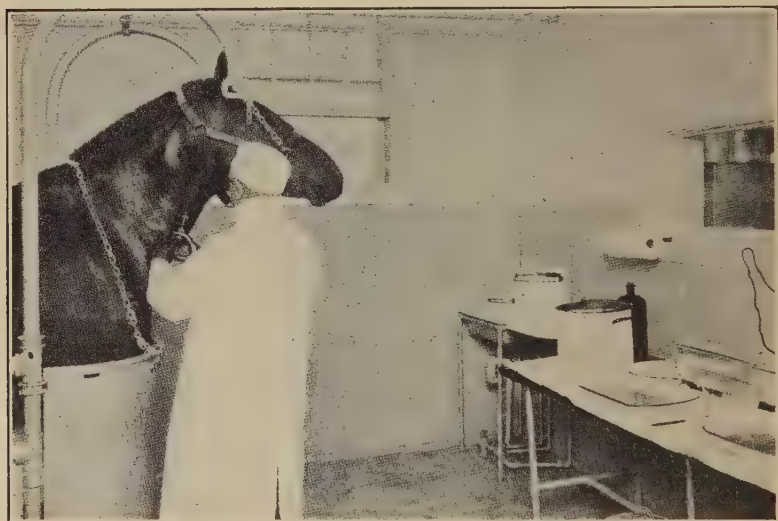


FIG. 129.—Injecting Serum Horses. (*Courtesy Parke, Davis & Co.*)

The injection of horses producing certain types of serum, such as those for meningitis and pneumonia, is made directly into the jugular vein, as shown in this picture. Horses producing antitoxin (diphtheria and tetanus), on the other hand, are injected subcutaneously in the neck or shoulder. In either case immunization is carried out with repeated administrations of increasing amounts of the antigenic material.

into a horse during the immunization process was sufficient to kill 500,000 guinea pigs weighing 250 grams each. Also, if antitoxin formation has gone on successfully, 1 cc. of the horse's blood serum will neutralize toxin sufficient to kill 50,000 guinea pigs weighing 250 grams each.

Collecting the Blood.—After the horses have reached such a condition where further injections of toxin are not accompanied by increase in antitoxic content of the blood, they are bled. The rooms in which this is done are usually apart from those in which the animals have been housed and received the toxin injections. These rooms are substantially constructed with apparatus and appointments similar to those in an operating room in a hospital. It is kept thoroughly aseptic and all instru-

ments and similar apparatus are carefully sterilized. The horse is bled by means of a sterile canula inserted into the jugular vein by means of a trocar. A gallon or more of the blood may be collected without injuring the animal. The blood is collected in sterile glass cylinders in which it is stored in refrigerators until it has clotted. The serum is then drawn off and treated with 0.4 per cent of tricresol. This was the antitoxin of commerce until Gibson and others devised methods of concentrating and purifying it. Some horses are such good antitoxin formers that

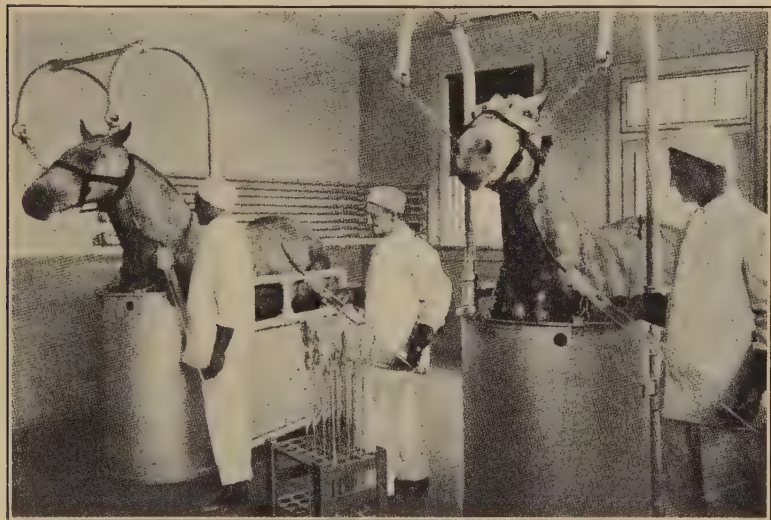


FIG. 130.—Bleeding Antitoxin Horses. (Courtesy Parke, Davis & Co.)

A canula introduced into the external jugular vein is attached to a rubber tube which in turn is connected with a glass tube entering the collecting receptacle. All procedures in the operating room are carried out with the same rigid regard for asepsis that would characterize surgical technique in a well-conducted hospital.

they may be bled over a long period. Others seem to be unable to produce antitoxins.

Measuring Strength of Toxins and Antitoxins.—The necessity of having antitoxin standardized to certain strengths is evident. Physicians must know how strong the product is else there would be little certainty that enough had been given. For this purpose the United States Public Health Service supplies standard antitoxin with which the new antitoxin may be compared. The method of testing may be briefly described as follows: The materials required are:

1. Diphtheria toxin.
2. Standard Antitoxin from United States Public Health Service.
3. The antitoxin to be standardized.

The toxin is first evaluated by means of the standard antitoxin. These are combined in such proportions so that when the mixture is injected into a 250-gram guinea pig there is enough free toxin to kill the pig in four days. The strength of the toxin does not vary but remains constant for a time. The same test is repeated except that the test antitoxin is substituted for the standard antitoxin. If the guinea pig used in this second test lives beyond four days the antitoxin is of sufficient strength. It is interesting to note that the government keeps standard antitoxin in Washington just as the standard yard measure. The standard antitoxin is necessary in order that there be available a constant unit.

Directions for Determining the Potency of Antidiphtheric Serum.—

These might be regarded by some as out of place in this book. They are included, however, to give some idea of the technic used in this work. A publication of Parke, Davis & Co. discussed the method as follows:

Things required

A bottle of Standard Test Serum diluted so that each cubic centimeter contains 1 antitoxic unit.

A quantity of diphtheria toxin.

Guinea-pigs, 250 grams each.

100 cc. volumetric flasks.

1 cc. bulb pipettes.

1 cc. pipettes graduated in 100s.

Combination bulb and graduated pipettes of 12 to 13-cc. capacity.

Watch-glasses with covers.

The first step in the process is to determine the L+ dose of the diphtheria toxin as follows:

One cubic centimeter of the test serum contains one unit of antitoxic potency. One cubic centimeter of this normal serum is placed in each of several watch-glasses. To each watch-glass is further added a certain amount of the diphtheria toxin, the amount varying from glass to glass to make a series, as for instance the following:

1.00	
.50	.20
.35	.15
.25	.10

These mixtures of toxin and antitoxin are then injected into guinea pigs, the mixture from one watch-glass being injected into one guinea pig. The guinea pigs should weigh 250 grams and should be in some way marked so that they can be distinguished one from each other. With an ordinary diphtheria toxin it is probable that those guinea pigs into which the larger of these amounts of toxin are injected will die, and that those guinea pigs into which the smaller amounts are injected will live, i.e., the smaller amounts of toxin will have been completely neutralized by the one unit of antitoxic potency. The time allowed for guinea pigs to die in this test is four days; guinea pigs dying after four days do not influence the results. We will suppose that

those guinea pigs receiving .25 cc. or less of toxin live, and those receiving .35 cc. or more of toxin die. In this case the L+ dose, or the least amount of toxin which will kill a guinea pig, and neutralize one unit of antitoxin, lies somewhere between .25 cc. and .35 cc.

To determine more accurately the location of this L+ dose, a second test may be made, similar to the above, by giving as doses of toxin the following:

.26	.31
.27	.32
.28	.33
.29	.34
.30	

In this case we may suppose that the guinea pigs receiving .29 or less of toxin will live, while guinea pigs receiving .30 or more of toxin will die. This determination would be accurate enough for all ordinary purposes, and .30 would be taken as the L+ dose of the toxin.

The second step is to determine from the L+ dose the antitoxic potency of the serum to be tested. In this case an amount of toxin corresponding to the L+ dose (in the example given above .30) is placed in each of several watch-glasses. To each watch-glass is further added a definite amount of the serum to be tested, the amounts being arranged so as to form a series, of which the following might be an example:

.005	.002
.004	.00166
.0033	.00133
.0025	.001

These mixtures of toxin and antitoxin are injected into 250-gram guinea pigs, the contents of one dish being injected into one animal. These are kept under test for four full days, then the records are made up, and the test closed. Any animal dying after this date does not affect the result. In this case, with commercial serum, it is probable that those guinea pigs receiving the larger amounts will live, and that, those receiving the smaller amounts of serum will die. We will suppose, as an example, that the guinea pigs receiving .002 or larger amounts of serum live, while those receiving .00166 or smaller amounts of serum all die; in other words, .002 (or $\frac{1}{500}$ cc. of serum is sufficient to neutralize the toxic effect of one L + dose of toxin, consequently $\frac{1}{500}$ cc. contains one unit while .00166 ($\frac{1}{600}$ cc. is not sufficient to neutralize the L + dose, consequently does not contain one unit of antitoxic potency, hence the serum under test contains at least 500 units per cubic centimeter, but does not contain 600 units per cubic centimeter. Should it be desired to determine more accurately just how many units are contained, another series of doses can be made out, running between these two limits.

In order to measure such quantities as .00166, .002, etc., accurately 1 cc. of the serum to be tested is first diluted to 100 cc. by placing 1 cc. in a 100-cc. flask and filling with physiological salt solution to the mark on the neck of the flask. Now, by using a 1-cc. pipette graduated in 100s for measuring out this dilution, very accurate readings can be obtained to one ten-thousandth of a cubic centimeter.

In these tests the death of the animal is the decisive factor. Other effects, such as swelling, necrosis, etc., are not determining factors.

Purified Concentrated Antitoxins.—Much study has been given the subject of separating the active constituents from the extraneous material in horse serum. It was believed that if the active constituents could be prepared there would then be no need of injecting into a patient's blood that portion which has no activity. By means of precipitation reaction such as those used by Gibson and others, the globulins which carry the antitoxins may be precipitated out. By means of the Gibson process, the antitoxic serum is treated with a saturated solution of ammonium sulfate which precipitates the globulins. The serum is discarded. The globulins after removal by filtration are redissolved in saturated sodium chloride. Acetic acid is then used for precipitating the pseudoglobulin, the euglobulin being valueless is discarded. The pseudoglobulin containing the antitoxic principles is collected and dialyzed in water to remove salts. A purified and concentrated antitoxic serum is thus obtained. This must be standardized by the same procedure outlined above. The preparation of this antitoxin has done much toward reducing the incidence of serum sickness.

Tests for Purity.—Following the potency tests are applied those tests which will show whether the product is safe to use and free from objectionable bacteria. Some of the serum is injected into guinea pigs to ascertain whether foreign toxins may be present. Then, tests are made for the presence of bacteria by inoculating some of the serum into suitable media. If the results of these tests are satisfactory, the product is considered safe. Every attempt is made to insure a product free from danger. The success of these attempts is indicated by the few accidents which have occurred in recent years.

Physicians who use antitoxic sera must have assurance that they are safe and of the proper strength else they will use them with a false sense of security. If a physician were well trained in chemistry and bacteriology and had a laboratory and the time, he could examine these products himself. Obviously, the best method is for the physician to be able to use the products without testing them and yet feel certain that the health of his patients will be protected. In 1902, by act of Congress the United Public Health Service was empowered to establish a system of control for vaccines, antitoxins, etc. In general, the procedure is to license certain manufacturers if they comply with requirements laid down by law and if their products meet certain standards. This standard is very high and the stamp of approval of the United Public Health Service is even sought by many foreign manufacturers even though they do not expect to compete with American manufacturers. At the close of 1926, thirty-six American manufacturers and eleven foreign establishments held American licenses to manufacture biological prod-

ucts. The federal government is not engaged in manufacture, only control. Before a manufacturer is licensed by the government a thorough inspection is made of the plant, personnel and equipment; his products are also examined by purchasing packages on the open market. In this manner the products are checked from two sides and the public may use them with great safety.

Use of Antitoxins.—The use of antitoxic sera may best be grasped by appreciating the position of a physician called to the bedside of a person ill with symptoms of diphtheria. Somewhat uncertain about

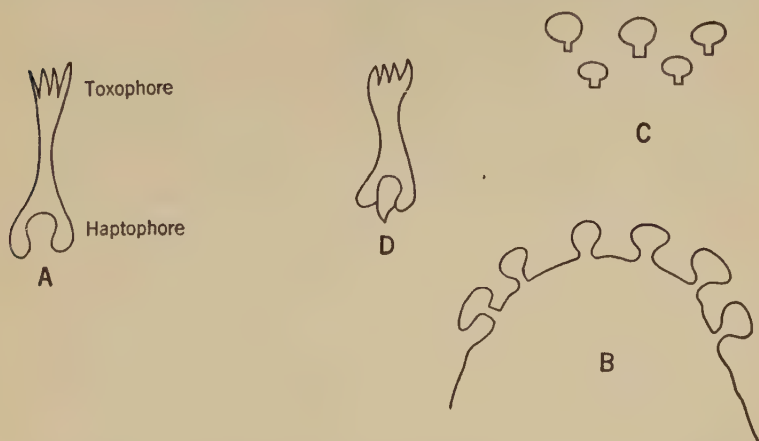


FIG. 131.—A Schematic Depiction of How Antitoxins Function in Diseases Like Diphtheria, Botulism, etc. According to Ehrlich's Theory.

A. A toxin molecule with a toxophore (poisoning) group and a haptophore (anchoring or binding) group. B. A body cell with a large number of receptors. These are eventually formed in such large numbers that they are thrown off of the cell and appear as free receptors in the blood stream. These are antitoxins. C. Free receptors in the blood stream. D. Showing a toxin molecule the haptophore part of which has been filled with a free receptor. This prevents this toxin molecule from uniting with a body cell to poison it. These free receptors are antitoxins.

the symptoms or wishing to confirm his clinical diagnosis with laboratory data, he will send a swab from the throat of the patient to the laboratory. On this swab will be carried a specimen of the membrane if one is present, or other material from the throat. In the throat of a child ill with diphtheria, the bacteria along with broken-down tissue, etc., constitute a diphtheritic membrane. The bacteria in this membrane grow and produce a soluble (extracellular) toxin which reaches the blood stream and is carried about the body. The body cells of the patient are poisoned and he may die from this toxemia. As soon as the toxin appears in the blood stream, the patient's body cells begin to make antitoxin. If they manufacture it rapidly enough to counteract the toxin

as it is formed the patient may get well. This is the case in a small percentage of cases. The physician, however, does not wait for this but decides to inject antitoxins which have been formed in the horse and not wait for them to be made by the patient. These antibodies or antitoxins are then ready to neutralize the toxin as it reaches the blood stream from the organisms in the throat. The action of antitoxins is explained in Fig. 131.

To be effective antitoxins should be injected as early in the disease as possible. Each delay of a day causes a higher mortality. Advanced cases demand high dosages and frequently, with due caution, intravenous injections. Public health authorities state that there is no reason for a single death from diphtheria, if parents are acute to the symptoms which appear and if antitoxin is administered promptly.

Allergic Reactions after Use of Sera.—After receiving injections of diphtheria antitoxin made in a horse, certain individuals who are sensitive to horse serum may suffer from serum sickness. This is characterized by rashes, enlargement of the lymph nodes, pyrexia, etc. The attack is not necessarily serious but is annoying. In some persons who are hypersensitive to the proteins in horse serum, the symptoms may be severe. This is another manifestation of anaphylaxis, other examples of which, have been discussed elsewhere in this book. Less trouble results from the use of purified concentrated antitoxic serum put out to-day, because, as we have discussed above, it has been freed from extraneous proteins and contains only those with which the antitoxins are connected. The above statements should indicate why physicians do not like to give antitoxins unless it is absolutely necessary. The promiscuous administration of antitoxic sera would result in a large number of sensitized individuals. Later on it might be necessary to administer antitoxin and they might suffer from serum sickness.

Even though a few deaths may have resulted from serum sickness following the administration of antitoxic serum, we should not overlook the great numbers of lives that have been saved.

Size of the Dose.—Antitoxins are standardized in units as discussed above. The size of the dose depends to a large extent on the symptoms of the patient, the progress of the disease, and the results which follow the first injection. When little improvement is observed after the first one or two injections, the size of the dose may be increased. Larger doses are also given when the diagnosis is delayed.

Reasons for Failure of Antitoxins.—Once in a while the administration of antitoxic sera has not been followed by desired results. Different explanations are possible. Among these, two may be mentioned as especially important. The antitoxic serum may be unsatisfactory on

account of either improper preparation or age. The other reason is late administration; the disease may have progressed to such an extent that the antidote (antitoxin) is without effect. Recent work has also shown that antitoxins must not be frozen since by freezing they are markedly reduced in potency.

Duration of Effect of Antitoxin.—How long do antitoxins remain in the blood after injection? Accurate data on which to base an answer possibly do not exist. About all that can be done is to quote the conclusions of the different investigators who have studied the problem. Undoubtedly, the persistence will depend on the size of the dose of antitoxin which is injected and the specific characteristics of the person receiving the injection. It has, in general, been found that antitoxins which have been formed in the blood of the same species as that into which the antitoxin is to be injected persist for a longer time. It would not be feasible, however, to prepare antitoxic sera in human beings.

The Schick Test.—This test has been well described in an article in the Public Health Reports of the United States Public Health Service, from which the following is quoted.

The Schick reaction is a convenient and reliable clinical test by which the antitoxic immunity of an individual to diphtheria can be determined. A fresh solution of diphtheria toxin is prepared for this purpose of such strength that 0.1 cc. or 0.2 cc. represents $\frac{1}{50}$ of the minimum lethal dose of toxin for 250-gram guinea pig. This amount is injected with a good syringe, preferably a 1 c.c. "Record" and a fine steel or platinum-iridium needle, *intracutaneously* on the flexor surface of the forearm or arm. A good guide for the insertion of the needle into the proper layer of the skin is to be able to see the oval opening of the needle through the superficial layers of the epidermis. A properly made injection is recognized by a distinct wheal-like elevation, which shows the prominent openings of the hair follicles. The result of the test should be read at the end of 24, 48, 72, and 96 hours.

The reaction that appears at the site of injection may be either positive, negative, pseudo, or combined positive and pseudo.

The *positive* reaction represents the action of an irritant toxin upon tissue cells that are not protected by antitoxin. It indicates, therefore, an absence of immunity to diphtheria. A trace of redness appears slowly at the site of injection in from twelve to twenty-four hours, and usually a distinct reaction in the course of twenty-four to forty-eight hours. The reaction reaches its height on the third or fourth day, and gradually disappears, leaving a definitely circumscribed scaling area of brownish pigmentation, which persists for three to six weeks. At its height the positive reaction consists of a circumscribed area of redness and slight infiltration, which measures from 1 to 2 cm. in diameter. The degree of redness and infiltration varies to a great extent with the relative susceptibility of the individual. The positive reaction is seen in about 7 to 10 per cent of the new-born, 30 per cent during the first year of life, 35 decreasing to 15 per cent between two and fourteen years, and 10 per cent of adults.

In the *negative* reaction the skin at the side of injection remains normal. The negative reaction definitely indicates an immunity to diphtheria if the test-toxin is of full strength, has been freshly diluted, and the injection has been made into the proper

layer of the skin. A negative reaction obtained in a child that has reached the age of three years indicates that it has an immunity which is probably permanent. Of 1000 carefully observed individuals, not one developed clinical diphtheria, even though they were exposed to the disease, and some were carriers of virulent diphtheria bacilli.

The *pseudo-reaction* represents a local anaphylactic response of the tissue cells to the protein substance of the autolyzed diphtheria bacilli, which is present in the toxic broth used for the test. Like other anaphylactic skin phenomena, the reaction is of an urticarial nature, appears early within six to eighteen hours, reaches its height in thirty-six to forty-eight hours, and disappears on the third or fourth day, leaving a poorly defined small area of brownish pigmentation and generally no scaling. At its height the pseudo-reaction shows varying degrees of infiltration, and appears as a small central area of dusky redness with a second areola, which gradually shades off

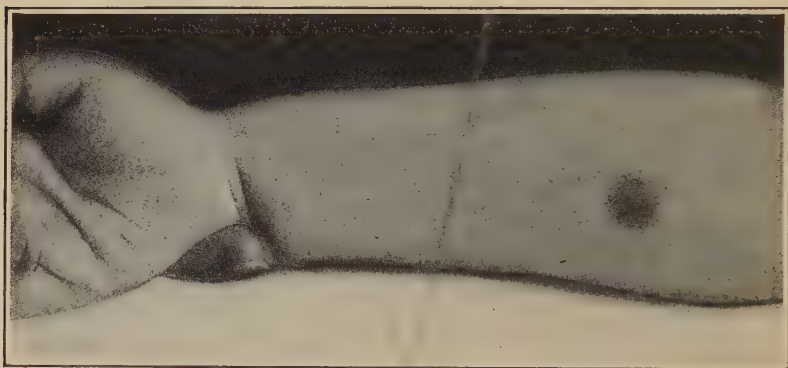


FIG. 132.—Showing the "Reaction" in a Schick Test.

into the surrounding skin. The reaction may also have a rather uniform red appearance, and be two or three times the size of true reaction. A control test, made by injecting toxin heated to 75° C. for five minutes, give a similar reaction which passes through the same clinical course. Individuals who give a pseudo-reaction only have antitoxin and are immune to diphtheria. The false reaction is seen in relatively few of the older children, but in much larger number of adults, in whom it is of importance to recognize and control it both by the injection of heated toxin and by observing the clinical course of the reaction.

The *combined* reaction represents the positive and the pseudo-reaction in the same individual. The central area of redness is larger and better defined, the amount of infiltration is also more marked. The reaction is recognized by noting the evidence of a true reaction, a definitive area of scaling brownish pigmentation, after the pseudo-element has disappeared in the test. In addition, a similar though weaker reaction is obtained in a control test made with heated toxin. The control represents only the pseudo-reaction. The combined reaction indicates an absence of immunity to diphtheria.

The Schick test is of practical value in determining the immunity to diphtheria of the public in general, but especially of the child population in schools, hospitals, institutions, and in homes during an outbreak of diphtheria. It will save a considerable

amount of antitoxin and avoid unnecessary sensitization of more than 65 per cent of the exposed individuals. The test is also of distinct value in the active immunization of susceptible individuals against diphtheria with mixtures of toxin-antitoxin, and in the diagnosis of clinically doubtful cases of diphtheria.—Text of a circular of information issued by the Department of Health, City of New York.

Toxin-Antitoxin Administration.—After learning that a person is susceptible to diphtheria, the next problem is to confer on him sufficient immunity to protect him from infection with an ordinary dose of bacteria. This may be done in different ways. It could, perhaps, be done by injecting some dead bacteria (a bacterial vaccine) or a small amount of the toxin formed by these bacteria. However, mixtures of toxin and antitoxin have been found to be especially useful for stimulating antibody formation. These mixtures are not equal mixtures in which there is just enough antitoxin to combine with the toxin; there is usually a very slight excess of toxin which stimulates the body cells of the individual receiving the treatment to make antitoxins. The toxin-antitoxin mixture is not a permanent union but a reversible one which tends to break up, forming free antitoxin, and free toxin. Consequently, this free toxin liberated in this manner acts as an antigen to cause formation of more antitoxin.

Immunization with toxin-antitoxin involves three injections of 1 cc. at weekly intervals. A person so treated is believed to be immune for years. This method cannot be used for the prevention of diphtheria among those who have been exposed. Several months are believed to be necessary for establishing a sufficient grade of immunity to protect.

The United States Public Health Service has discussed the use of toxin-antitoxin as follows:

1. *As a general prophylactic measure.*—The most suitable age period for testing and immunizing is between six months and two years. At this time of life the percentage of positive Schick reactions is largest and the susceptibility to diphtheria as well as the mortality from the disease is greatest. Children of this age period can be reached in the homes, in infant asylums, in the milk stations, and day nurseries. The children of the next age period are included in the preschool groups. These children can be reached in the public schools, in orphan asylums, and in the various other institutions. Among adults, those who come in contact with diphtheria and are constantly exposed and in danger should also be tested and immunized with toxin-antitoxin if found to give a positive Schick reaction. Included in this group are especially physicians, nurses, and hospital attendants in contagious-disease hospitals.

2. *To control an outbreak of diphtheria.*—As the immunity arising from an injection of toxin-antitoxin does not develop until the lapse of from two to twelve weeks, active immunization cannot be utilized to protect persons from exposure within that period. In institutions, however, where small outbreaks of diphtheria have occurred, or where diphtheria is more or less constantly present and clinical cases and bacillus carriers steadily appear, the use of antitoxin alone has often been insufficient to stamp

out the disease, but the combined application of the Schick test and active immunization with toxin-antitoxin has given successful and encouraging results. Toxin-antitoxin immunization should not be used with antitoxin immunization in the same individual, as the surplus antitoxin tends to prevent the development of an active immunity.

Different Kinds of Antitoxins.—Besides the diphtheria antitoxin which has been used as a type example for antitoxins in general, there are several other kinds which have been made with the anticipation that they would be useful. In each case the organism causing the toxemia, for which the antitoxin is the antidote, forms a soluble toxin.

Botulinus Antitoxin: Botulism, an acute toxemia, is caused by a soluble toxin formed by *Clostridium botulinum*. It has been of special significance in foods. Botulinus antitoxin is prepared by the same procedures which have been outlined for diphtheria antitoxin. The horse is used on account of certain characteristics. Since there are three races or strains of *Clostridium botulinum* known as Types A, B, and C, it is necessary either to use a mixture of the three types in the antigen with which antitoxin formation is stimulated in the horse or to use the separate toxins on different horses, and find out what type of antitoxin is necessary before it is administered. The latter procedure would be time-consuming and time is very important in the administration of antitoxins, especially in botulism. Owing to the inherent characteristics of botulism and the fact that diagnosis may be delayed until the intoxication has progressed until the symptoms allow a diagnosis, the same success has not followed the administration of this antitoxin that has followed the use in diphtheria.

Tetanus Antitoxin.—Antitetanic serum is prepared by injecting horses with tetanus toxin in the same manner as has been outlined for diphtheria antitoxin. A slightly different situation exists, oftentimes, in the administration of this serum in tetanus than exists when diphtheria antitoxin is given in diphtheria. Tetanus antitoxin may be administered after a person has suffered a deep wound which has been contaminated with considerable dirt. Its purpose is that of a prophylactic or preventive agent to be used before there are symptoms. It is argued that such dirt may contain tetanus spores which will germinate in the blood to cause subsequent intoxication. Antitoxin in such cases usually prevents subsequent poisoning. Delay in the administration of tetanus antitoxin until the symptoms have appeared results in a much higher mortality than is the case where the antitoxin is given to prevent potential toxemia. The administration of tetanus antitoxin has become a well-established practice.

Available information seems to indicate that the antitoxin does not

persist in the blood very long. The immunity is therefore passive and of significance only quite soon after its injection.

Gas Gangrene Antitoxin.—This antitoxin is prepared in horses with the toxin of *Clostridium welchii* as the antigen. It is used in the same manner as are other antitoxins. It has been especially useful in treating the toxemias resulting from infection of war wounds with *Clostridium welchii*.

Scarlet-fever Antitoxin. *Streptococcus scarlatinae* is now generally accepted as the etiologic agent in scarlet fever. Many years of research were necessary to produce convincing evidence. The work of Dr. George H. Dick and his wife, Gladys Dick, has resulted in not only the general acceptance of the above-named organism but in the development of an antitoxin which is regarded by many to be quite satisfactory as a curative agent. This antitoxin, of course, has not received the study has been given the older and better known ones and it may be that future work will indicate it to be of greater or less value.

Dick Test in Scarlet Fever.—This test for scarlet fever susceptibility simulates very closely the Schick test for diphtheria. It consists of an intradermal injection of from 0.1 to 0.2 cc. of a dilution of the soluble toxic filtrate obtained from a culture of a specific hemolytic streptococcus. To secure the toxin, the organism is grown for several days in broth containing a little horse blood. Positive Dick reactions, which indicate a susceptibility to scarlet fever, are characterized by a reaction quite similar to the reaction in a positive Schick test. According to Zingher,¹ however, the reaction appears more promptly.

For immunizing those who are shown to be susceptible by the Dick test, scarlet fever toxin in small amounts is used. This causes the appearance of an active immunity. Small amounts of toxin are used at first. Subsequent injections consist of larger doses as the individual becomes accustomed to them and his resistance is increased.

Directions for Determining the Potency of Antitetanic Serum. The following directions are quoted from a publication of Parke, Davis & Co.:

The same apparatus will be needed as mentioned in directions for carrying out the test for antidiphtheric serum.

The test dose (L + dose) of the standard tetanus toxin issued by the Hygienic Laboratory, U. S. Public Health and Marine Hospital Service, is 0.000,6 gram.

This test dose was 100 MLDs. The MLD of this toxin was 0.000,006 gram.

Although the standard unit is based upon the neutralizing power of 100 MLD of this test toxin, the number of MLD will no longer be a factor in the question any more than it is in similar work with diphtheria. The neutralizing power of an arbitrary quantity of the standard tetanus antitoxin preserved in Ehrlich vacuum apparatus

¹ Zingher, A. The Dick Test and Active Immunization with Scarlet Fever Toxin. Public Health News, New Jersey Dept. Health, 9 (1924) 320-329.

under special conditions in the Hygienic Laboratory is the unit. This quantity of antitoxin was originally determined against 100 MLD of the standard toxin A. So that hereafter the test dose of the toxin which is issued will be spoken of as the Test Dose of the L + dose and is not necessarily 100 MLD.

The unit of tetanus antitoxin is ten times the quantity of serum necessary to save the life of a guinea pig four days (96 hours) against the test or L + dose of toxin. The antitoxin and toxin are mixed, allowed to stand one hour at room temperature before injecting subcutaneously into the guinea pig. The guinea pig should weigh about 350 grams.

The standard tetanus toxin for making these tests is sent out in small vials and should be kept dark, cold, and in a vacuum desiccator over P_2O_5 or H_2SO_4 . Under these conditions it will show little change in the course of months.

The best way to dissolve the standard tetanus toxin for the purpose of testing is as follows: Carefully tare a weighing bottle, then add approximately 20 or 30 mg. of the dry poison. Again carefully weigh. Now dissolve the toxin directly in the weighing bottle with physiological salt solution (.85) in the proportion of 0.1 gram of the dry toxin to 166.66 cc. of the salt solution; this proportion is used for the reason that each cubic centimeter of this solution will represent 0.000,6 gram of the original dry poison. The proportion is taken because it is very convenient in measuring out the test dose. Example:

$$\begin{array}{r}
 44.5692 \text{ gm., Bottle + toxin} \\
 44.5300 \text{ gm., Bottle} \\
 \hline
 .0392 \text{ gm., Toxin} \\
 0.1 \text{ gm. : } 166.66 \text{ c.c. :: } 0.0392 : x \\
 x = 65.33 \text{ c.c.}
 \end{array}$$

In other words, if the quantity of toxin placed in the weighing bottle should weigh, as in this instance, just 0.0392 gm., carefully deliver from an accurately graduated burette just 6.533 cc. salt solution into the weighing bottle; and, as before stated, each cubic centimeter of this solution will be the L + or test dose. The toxin supplied is of very nearly the same strength as that supplied by the U. S. Government and can be used for test purposes according to the directions just given. See statement of quantity on the label of the bottle and make dilution accordingly.

AGGLUTININS

The agglutinins are antibodies which agglutinate or clump their antigens. The antigens in this case are usually bacterial cells. Agglutination takes place in two stages. First of all there is a loss of motility and later a clumping of the bacterial cells. Both of these phenomena are observed by bacteriologists.

Preparation of Agglutinins.—These antibodies are formed in the blood of animals. They are formed by stimulation with an antigen and are specific in their action, i.e., agglutinins formed by the injection of one antigen will not react with another antigen. The antigen may be injected into the veins or body cavities of the animals. The bacterial

cells may be dead or alive; if dead, they should have been killed by heating to temperatures below 62° to 65° C. Destruction at temperatures above this range causes the cells to lose their antigenic value, or power to stimulate the production of agglutinins. If rabbits are used, the bacterial cells may be injected into the ear vein; the blood of the rabbit may be frequently tested to make certain its blood contains sufficient agglutinins, before the rabbit is finally bled. If satisfactory, the animal is bled aseptically. The blood is allowed to clot and the serum removed and treated with tricesol, which acts as a preservative. It is then stored in a refrigerator.

Demonstration of Agglutinins.—Agglutination reactions are carried out by mixing some blood serum from an animal which has received injections of bacterial cells as antigen, with some of these cells and then examining the mixture under the microscope for the presence of clumping or agglutination. The blood serum may be diluted even two or three hundred times and still show its specific action. The reaction is characterized by a loss of activity by the cells and a drawing together into clumps. There are usually few free bacteria in the field. The laboratory worker usually makes a control preparation—one which contains the organisms without any added antiserum containing agglutinins. This preparation is made to determine whether there is any tendency to spontaneous agglutination of the cells in the culture and serves as a control with which to compare the unknown preparation.

The serum containing the agglutinins is usually diluted to overcome the influence of *normal agglutinins*. These are agglutinins which are present in the blood of normal persons.

Practical Application of Agglutinins.—The specificity of the agglutination reaction has caused it to be turned to very useful applications, only two of which will be considered here. The basic principles of agglutination may be found in the more advanced texts.

The Gruber-Widal Reaction.—This is an application of the agglutination reaction to the diagnosis of disease. Its use in the diagnosis of typhoid fever was established by Widal, after whom the test is named. The test may be more easily understood if one imagines himself in the place of a physician who is called to the home of a person who is ill with symptoms of typhoid fever. These may not be quite characteristic and he may desire the aid of laboratory data to confirm his diagnosis based on clinical data. The physician knows that, if his patient has typhoid fever, *Eberthella typhi* is present. The presence of *Eberthella typhi* will stimulate the production of agglutinins in the patient's blood. Consequently, if agglutinins can be shown to be present, the physician infers the presence of *Eberthella typhi* and probably typhoid fever. In order

to find out whether these agglutinins are present, he takes a small portion of blood from the patient and sends it to the laboratory. The laboratory is supposed to determine whether or not the blood contains agglutinins for *Eberthella typhi*.

The blood may reach the laboratory dried on a slide or in a small tube. If not dried, it is brought back to about its original volume and further diluted in order to avoid agglutination which may result from the presence of normal agglutinins. This may require dilution of one part of serum with 50 or 60 parts of sterile physiological sodium chloride solution. Then to a drop of this diluted serum are added some living cells of *Eberthella typhi*. If agglutinins are present, these cells will be agglutinated or clumped after an hour at 37° C. The presence or ab-



FIG. 133.—A Schematic Depiction of an Agglutination Reaction.

A. Freely moving bacteria in a microscopic field. B. Another preparation from the same culture with the exception that specific agglutinating serum has been added. The bacteria are agglutinated.

sence of agglutination is reported to the physician. This constitutes the Widal reaction or the so-called "blood test" in typhoid fever.

The interpretation of the Widal or agglutination reaction is influenced by the fact that a person may have received preventive inoculation against typhoid fever; the agglutination response may thus have resulted from agglutinins from prophylactic inoculation and not from a natural infection. This possibility must be considered by the physician whose duty it is to weigh the evidence, both clinical and laboratory, before he announces his diagnosis. Although agglutinins are formed during infections, there is considerable evidence that the agglutination titer cannot be taken as an index of the amount of immunity.

Identification of Bacteria by Agglutination.—In the Widal reaction just described, two main factors were concerned, cells of *Eberthella typhi* and blood serum from a person suspected of typhoid fever. The unknown factor was the agglutinins in the patient's blood. It is obvious that the same agglutination reaction could be made with the bacterial

cells as the unknown factor. In this case, a known anti-serum prepared from the blood of some animal would be used. This antiserum would be made by injecting a rabbit for instance, with a pure culture of a microorganism. The blood serum would be collected and used to agglutinate the unknown microorganism. This allows us to run the Widal reaction backwards, so to speak, and instead of trying to detect the presence of agglutinins to use known agglutinins to identify unknown bacteria. This test is the final one usually applied for the identification of an organism after the morphological and physiological tests have been determined.

Agglutination Tests in Blood Transfusion.—This is discussed at this time because it represents another type of agglutination and because it answers a question frequently asked by students of bacteriology. Transfusion of blood is frequently necessary. It has been resorted to in injuries where great amounts of blood have been lost, such as war wounds, hemorrhages, etc. The two principals involved in blood transfusion are the patient, who is spoken of as the recipient, and the donor who gives the blood. Before taking blood from an individual (the donor) it is necessary to determine whether his blood is compatible with the blood of the recipient. To determine this, tests are made to determine whether the red blood cells of the donor are agglutinated by the blood serum of the recipient or patient. If they are agglutinated or hemolyzed, this donor cannot be used, for his blood would not benefit the patient; it might even harm him. Bloods from different individuals have been classified by investigators into groups, the best known is that by Moss. We need not present here the definitions of these groups. They are given in advanced texts and are usually verified experimentally in advanced courses in practical bacteriology.

PRECIPITINS

The precipitins are antibodies which precipitate their antigens. The relation of precipitins to immunity is not understood. They are known only by their reactions, which are among the most interesting of serological reactions. Their action is specific and their property gives them their significance in bacteriology.

Preparation of Precipitins.—These immune bodies are also prepared in the blood of some animal. On account of the cost and ease of handling, the rabbit is generally used. The antigen is injected at intervals of three to five days, usually in the peritoneal cavity. The body cells of the rabbit make precipitins against the antigen. As stated in a subsequent paragraph, the precipitins are used especially for the detection

of human blood. In this case antisera (or precipitating sera) are made by injecting the blood of various species into rabbits.

Practical Applications of Precipitins.—Since the action of precipitins has been shown to be specific, they are used for the detection and identification of proteins. They may be used for the identification of blood, the detection of horse meat in sausage, the separation of natural from artificial honey, etc. While the precipitin reaction is a very delicate one, it has certain limitations. It cannot be used for separating proteins (blood) of closely related animals such as the cow and goat, sheep and goats, man and other primates, etc.

Detection of Human Blood.—A very interesting forensic use of the precipitins has been connected with the identification of blood spots on the garments of suspected murderers. Before the precipitins were known, the biochemical methods of blood identification were often unsatisfactory. Chemical methods may be used to prove that certain spots are blood spots while the precipitin reaction will identify the blood as that from a certain species.

The following quotation is from a report by Doctor Stokes ² of the use of the precipitin reaction for the identification of blood:

The first case in which the precipitin test for human blood was used was of the State of Maryland vs. Norman Mable, and James Parroway, which was tried in the circuit court for Cecil County on Wednesday, the 4th of March, 1914. The testimony showed that a brutal murder had been committed upon a well-known citizen living near Salisbury in Wicomico County. In travelling from the country store to his home the victim was waylaid by the two men and struck over the head several times with a corn planter, the assault resulting in the death of this man. A great deal of blood flowed from the wounds of the head, and some of the garments of the suspected murderers were brought to the laboratory for examination. A spot of blood on a button of the left sleeve of the coat of one of the murderers was detected by means of the usual chemical and micro-chemical tests, and 0.4 of a milligram of the blood was scraped from the button and accurately diluted so as to make a dilution of 1-1000, 1-10,000 and 1-20,000. The first dilution was also used as a color comparison for the second test in which no accurate weighings could be made. A spot of blood was also detected on the overalls of one of the murderers, and this spot was cut out and soaked in 2 cc. of salt solution and then diluted to the color of the weighed solution from the button, which equaled a dilution of 1/1000. Other dilutions equaling 1/10,000 and 1/20,000 were then made from this original dilution.

The technic, as described above, was then carried out, including the con-

² Stokes, W. R. The Use of the Precipitin Test for the Detection of Human Blood in Criminal Trials. Bost. Med. and Surg. Jour. 1917.

trol tests recommended, and the dilutions of 1/1000 of suspected human blood, as well as the controls, gave a distinct clouding within about two minutes and were distinct at the end of thirty minutes. The higher dilutions of 1/10,000 showed a beginning precipitum in about five minutes and was well marked at the end of thirty minutes. The tests were made at the usual room temperature.

The murder was committed for the sake of robbery and about fifty dollars were taken from an envelope found in the inside coat pocket of the victim. His coat was extensively stained with blood, and the envelope addressed to him was found at a short distance from the site of the crime. Dilutions of 1/1000, 1/1,000 and 1/20,000 were made by soaking the blood-stained paper of the envelope in salt solution and comparing the lowest dilution by color to the weighed solution of dried blood of 1/1000. The dilutions of 1/1000 and 1/10,000 gave a positive precipitin test, as described above.

In giving the testimony it was admitted that the test might be positive if the blood had been from some of the higher order of apes, but with that exception the opinion was given that the test was positive for human blood. The finding of blood stains on the button of the coat and the overalls of one of the suspected criminals was considered as important evidence, and the man was convicted of murder and received a long-term sentence, but was not hanged.

This murder had stirred up great feeling in the town of Salisbury and a mob attacked the jail and attempted to remove the prisoners. The attempt was thwarted, however, and the prisoners were removed to another county for safe keeping and trial.

While public opinion was still seething, a woman living just outside of Salisbury reported to the police authorities that while alone in her house she had been attacked by a man, who had then made away with a few trifling articles, having not been able to find any money or valuables. Upon investigation of the doorpost, the threshold of the door, a satchel and various other articles were found smeared with blood and the woman claimed that she had defended herself with a knife and had inflicted certain wounds upon the man, who then escaped. It should be mentioned that the woman was married, that her husband was absent from home and had been used to leaving her in the house in a lonely part of the country upon various other occasions. The wood from the door, the blood-stained satchel, a blood-stained corncrib, and a blood-stained knife were brought to the laboratory for examination, and the usual tests soon demonstrated the presence of blood upon all of these materials. Some of the blood, however, was dissolved in normal salt solution and examined. Somewhat to our surprise it was found that a number of the shriveled corpuscles contained nuclei and this was confirmed by stained specimens. It was thought probable that the blood might be from a chicken, and rabbits were immunized with chicken blood. When the blood serum of an immunized rabbit had attained a strength sufficient to produce a precipitation in a dilution of 1/20,000, the blood from these various materials was tested in the usual dilutions of 1/1000, 1/10,000, and 1/20,000. They all gave a marked reaction with the blood serum of the rabbit immunized with chicken blood,

and the opinion was then expressed that the blood did not come from a human being but was chicken blood.

There had been a great deal of excitement amongst the public concerning the second alleged criminal attack but when the woman was confronted with the evidence she admitted that she had staged a murder in order to keep her husband at home, as she thought that such an experience might make him more apprehensive of her safety in the future.

No further attempts, therefore, were made to apprehend the criminal, the public excitement subsided, and the husband presumably no longer strayed from his own fireside.

Foreign Proteins in Foods.—The precipitin test may be used for detecting sophistication in foods, such as substitutes for natural honey, the use of egg substitutes in pastries, presence of horse meat in sausage, as stated before. This application of the test is not so important in America where there is probably less substitution of one meat for another, but in some of the foreign countries, where horse meat is sold, the test is useful. The precipitin test in this case has some limitations; it is impossible, for instance, to separate the flesh of the cloven-hoofed animals or species which are very closely related in structure.

Separation of Species of Animals.—The precipitin test has also been used for the study of the origin and relationships of different species of higher animals. The test, therefore, has value in bringing useful data to bear on the discussion of evolution. It is quite well established that the precipitin test will not separate the proteins (in blood or tissue) of closely related animals. For instance, it has just been stated that the flesh of the cloven-hoofed animals cannot be separated. Boyden³ has stated that the building of family trees has practically ceased to-day not because the problems of animal relationships have been settled but because scientists are helpless. There is a general agreement as to the relationship of animals within certain main branches but not as to affinities of many of the invertebrates to each other or to vertebrates. Boyden³ pointed out that our present classifications and groupings are based on morphological data; consequently it may be wrong to attempt to make the serological reactions follow the morphological. Man cannot be separated by the precipitin reaction from other primates and this may indicate a deeply seated relationship.

Uhlenhuth, who did some of the pioneer work with the precipitin reaction, has recently stated that the absence of precipitin formation to a foreign blood is a fine indicator of biological relationship. It is impossible to induce the formation of precipitins by the injection of

³ Boyden, A. A. The Precipitin Reaction in the Study of Animal Relationships. Biol. Bull. 50 (1926), 73-108.

asses' blood into horses and vice versa. Rabbits may produce precipitins to hares' blood. Uhlenhuth, therefore, believed that hybridization of hares with rabbits was impossible. Some of the lower species of monkeys produce precipitins to human blood.

OPSONINS

Opsonins are antibodies (immune bodies, protective substances) which prepare their antigens (bacterial cells) for ingestion by the leucocytes or phagocytes. The phenomenon of ingestion of the bacterial cells is spoken of as phagocytosis or opsonification. The antigen (bacterial cells) is altered in some manner making it easier for the phagocytes to ingest them. When the bacterial cells have been taken into the phagocytes, they are dissolved, probably by enzymes. Opsonins are of significance in connection with the theory of immunity proposed by Metschnikoff.

Normal and Specific Opsonins.—Normal persons (those who have not had an attack of disease or received preventive inoculation) have opsonins naturally present in their blood. Such opsonins are, however, quite often non-specific, showing no special affinity for any one micro-organism. In contrast with these normal opsonins are those which appear in the blood after infection or preventive inoculation. Such opsonins are specific, showing marked affinity for the antigen (bacteria) which stimulated their formation.

Opsonic Index.—The determination of the opsonic index is based on a desire to know the opsonic power (or opsonin content) of the blood of an individual who has either suffered an attack of disease or received preventive inoculation. Instead of spending more time in discussing this determination, it might be more readily understood by outlining briefly the method for determining the opsonic index. This is taken from a publication of Parke, Davis & Co.

The opsonic index is the ratio between the average number of bacteria found within 50 to 100 leucocytes in a suspension containing bacteria, blood, corpuscles and the patient's serum, and the average found in the same number of leucocytes in a corresponding suspension containing normal serum, the latter being taken as the standard.

In a large number of infections, protective substances (opsonins) exist in the blood serum. The opsonins are thermolabile; the serum containing them is inactivated (that is, deprived of its opsonins) if heated to 56° C. for a few minutes. The opsonins act upon the bacteria, so that the latter can be subsequently ingested by the leucocytes. When the different blood specimens are compared, the variable factor is the serum and not the leucocytes. The specific opsonin is consumed when bacteria are added to a serum containing it; for, if the bacteria are then removed and the serum used with a second portion of the same suspension, it is found to be inactive. The

opsonins become combined with or at least absorbed by the bacteria so that bacteria removed after treatment and placed in an inactivated serum are freely taken up by the leucocytes added to the serum. By careful vaccination with small measured quantities of dead cultures of various pathogenic microorganisms, it is possible to increase markedly the opsonizing power of the serum of an individual. Regarding phagocytosis as the main process by which bacteria are destroyed within the organism, and the opsonins as the means whereby the bacteria are prepared for ingestion, Wright concluded that the relative amount of opsonins in a given serum affords an indication of the defensive power of the individual.

The technic of taking the opsonic index may be described in the following stages:

1. *Collecting the Blood to be Tested.*—By pricking the finger or the lobule of the ear the blood is obtained. It is drawn into a Wright capsule or a Widal tube, and the container is sealed with wax. One capsule of known normal blood is drawn. The capsules are numbered for identification, and left until the serum separates, or it may be centrifugalized to save time.

2. *Preparing the Blood Corpuscles.*—A few drops of blood from the finger are run into a test-tube which contains sodium citrate solution, $1\frac{1}{2}$ per cent, until the proportion is about two-thirds citrate solution and one-third blood. The contents are then gently mixed and centrifugalized for three minutes. The fluid is pipetted off, and normal salt solution is added to the corpuscles. The centrifugalizing is repeated, and the fluid again pipetted off, leaving only the layer of red and white corpuscles in the tube; with a pipette these corpuscles are gently mixed, and are now ready for use.

3. *Preparing the Suspension of Bacteria.*—The organisms, grown on agar, are removed, and suspended in salt solution. If necessary to break up clumps, the culture should be shaken. The suspension is centrifugalized, pipetted off into a fresh tube, and thoroughly mixed. It should then be examined as an ordinary smear preparation to be certain of its freedom from clumps.

The salt solution used in the suspension is 0.85 per cent saline, except in the case of tubercle bacilli and the non-gram-staining cocci, for which a 1.5 per cent saline solution is used.

4. *Combining for the Test.*—With a pipette having a long stem, equal quantities of the washed blood corpuscles, the bacterial suspension, and the serum to be tested are taken up, driven out on to a slide to be thoroughly mixed, and redrawn into the pipette; the end of the pipette is then sealed in a flame, care being taken to keep the suspension away from the heated end. The pipette is then incubated at $37^{\circ}\text{C}.$: in the case of typhoid and other coliform organisms eight to ten minutes is quite sufficient, and for other organisms fifteen to twenty minutes.

5. *Preparing the Film.*—On removing the pipette from the incubator, the sealed end is broken off, and some of the suspension expressed upon a slide and thoroughly stirred. A drop of this mixture is then placed on another slide, and spreading it with the slightly concave edge of an ordinary slide a film is made in which all the white corpuscles are swept to one end, where they are fixed and stained with the appropriate staining fluid.

6. *Counting.*—The microorganisms in 100 polymorphonuclear leucocytes are counted. The average number of bacteria per leucocyte is the phagocytic index. The consistency of the suspension of bacteria should be such as to facilitate a ready enumeration of the microorganisms in each leucocyte.

The quotient of the number of organisms in 100 leucocytes from the patient's serum, divided by the number in 100 leucocytes from the normal serum, is the opsonic index.

The opsonic index may be used as the controlling factor in the administration of vaccines.

Determinations of opsonic index are less important to-day than formerly. Kolmer stated that the opsonic power of a normal person to most pathogenic bacteria varies from 0.8 to 1.2. Opsonic determinations, theoretically at least, should be of assistance in vaccination for determining the size of the doses of vaccine and the best time for their administration.

LYSINS

The lysins are agents which destroy cells. In the blood they destroy bacterial cells and are spoken of as *bacteriolysins*. In a like manner *hemolysins* destroy red blood corpuscles (erythrocytes). Normal blood from subjects who have not had disease or received preventive inoculation is known to be germicidal. This property is best explained on the basis of its lysin content. Such lysins, however, are not specific but may destroy all bacterial cells. Specific lysins may be built up in the blood by some special treatment such as an infection with pathogenic bacteria or by inoculation with a bacterin. As with all immune bodies, lysins are specific and therefore probably play a rôle in immunity,

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CHAPTER XXXI

THEORIES OF IMMUNITY

As information slowly accumulated in medical science, it was observed that a person acquired an ability to resist a disease by having it or receiving preventive inoculation. These observations required explanation and several theories were advanced. Some of them are of historical interest only; they were accepted for a time but were discarded when better explanations were given, or when they were inadequate to explain new facts. In many cases these old explanations were reasonable and fitted well into the structure of knowledge of the time. The first two discussed below were probably based on the older theory for explaining cessation of growth on culture media.

Exhaustion Theory.—According to this explanation of immunity, which seems to have been suggested by Pasteur, some essential substance was necessary for the growth of pathogenic bacteria in the human body. This substance was believed to be present only in certain amounts and not to be regenerated anew. When it was *exhausted* the etiologic agent causing the infection was unable to grow. Consequently reinfection could not occur.

Noxious Retention Theory.—This was, to a certain extent, just the opposite of the exhaustion theory. Instead of some necessary substance being used up, the etiologic agent excreted a substance which accumulated in the subject of infection. This substance was believed to be a noxious excretory product which was inimical to the organism causing the infection. Finally the concentration of this substance became so great that recovery resulted, to be followed by immunity.

Metschnikoff's Theory of Immunity (Phagocytosis).—This theory is founded on the activity of the white corpuscles in the blood. In grammar school physiology one is told that the white corpuscles, or leucocytes, are the policemen or scavengers of the blood stream. They stand ready to give battle to invading forces which would cause disease. The white corpuscles which act in this manner are called phagocytes and the phenomenon phagocytosis. The phagocytes ingest the invading bacteria and digest them. Metschnikoff found it difficult to fit some new

discoveries into his theory; this kept him busy making new explanations and seeking new data.

Ehrlich's Side-chain Theory.—Another theory of immunity was proposed by Ehrlich, a German biochemist and bacteriologist. He was opposed to the theory propounded by Metschnikoff and caused the latter considerable difficulty. Ehrlich's theory became known as the side-chain theory and was based on observations which Ehrlich made in other fields. The theory was outlined as follows in a publication of E. K. Mulford & Co.

In biochemical structure cells consist of two parts: a nucleus upon which depends the nature and properties of the cell, and a great number of side chains or receptors by means of which the nucleus enters into chemical relation with substances reaching it through the circulation, as foods, etc. When an infection or poisoning occurs, toxic substances are distributed throughout the blood stream and are taken up by the receptors for which they have suitable chemical side chains. That this actually occurs is clear from the fact that certain poisons attack only certain cells, while others attack all the cells, and also by the fact that some substances are toxic for one species of animal and not for another, on account of lack of receptors to attack the poisons to the animal cell. Lack of receptors is therefore a form of natural immunity against toxin.

Ehrlich's theory of *cell receptors* offers a hypothesis both for the cause of disease and for its cure. The incubation period of an infectious disease is, apparently, only the time necessary for the formation of receptors for a substance that has entered the blood for the first time. When the receptors are formed and the poison begins to act on the cell nucleus, a reaction takes place, and either the cell itself is destroyed or the receptors with the poison attached are thrown off. If the cell survives and the receptors are thrown off, new specific receptors are formed and enter the blood stream. Having lost their connection with the cell nucleus, when combined with the toxins in the blood, their effect is to render these toxins harmless, and they are eliminated from the organism as waste products. This theory is further supported by the fact that the incubation period of infectious diseases is shortened with each succeeding infection. It is possible that animals with no specific receptors for a given toxic agent would become susceptible (receptors would be formed) if given extra large or continuous doses of the agent for a long time.

A very instructive illustration is found in the action of tetanospasmin, the constituent in tetanus toxin that causes muscular spasm. As the toxic properties of this poison are apparently exercised on the nerve cells alone it is evident either that these cells are the only ones with receptors for this substance, or that all body cells have receptors, but that the nerve cells are the only ones susceptible of being injured by this poison. As tetanospasmin is toxic for the nucleus of the cell, when it becomes attached by means of the cell receptors

there is a reaction between the nucleus and the poison which results either in the destruction of the cell or the casting off of the poison with its receptor. New receptors are formed and in such excessive numbers that they are continuously cast off into the circulation. They still retain the power of combining with tetanospasmin, but have lost the power of combining with the cells; they thus neutralize the toxin before it reaches the cell. Continuous casting off of the receptors represents a weakening of their power of attachment to the cell proper, and this in itself would constitute a very effective immunity reaction.

According to Ehrlich, poisonous substances consist of two chemical groups or portions, the poisonous group and the combining group. The first is known as the toxophore group and the second as the haptophore group. The receptors likewise have distinct functions, and, according to these functions, have been classified by Ehrlich into three groups. The first group comprises the antibodies against toxins and ferments (antitoxins and anti ferments). These possess only one binding group, which has an affinity for the haptophore group of toxins and ferments. They are called haptines of the first order. Haptines of the second order possess, besides the haptophore group, by means of which they attach themselves to the bacteria or other substances an agglutinophore and precipitinophore group by means of which they produce agglutination or precipitation. Haptines of the third order are the amboceptors which, in addition to their haptophore group, possess a complementophore group or ability to unite with complement. Bacteriolysis is explained by Ehrlich as being due to a substance formed in the process of immunization which has two binding groups. One of these binding groups has an affinity for the bacterial cell and is called the cytophile group, the other has an affinity for complement and is known as the complementophile group. Bacteriolysis takes place as follows: The cytophile group attaches itself to the antigen (bacteria) while the complementophile group attached itself to the complement. The complement is a sort of digesting (proteolytic) ferment. While present in the blood under normal conditions it does not act on bacteria without the aid of the amboceptors (bacteriolysins). Once this union has taken place the bacteria are dissolved by the complement.

Vaughan's Theory.—This explanation of immunity has not received the study and advertising that previous theories enjoyed. An understanding of Vaughan's theory presupposes an understanding of his conception of the structure of the protein molecule. Vaughan explained immunity to disease on the basis of the presence of certain enzymes which are formed in body cells. The immunity which results from an attack of disease was explained on the basis of the presence of an enzyme which was formed during the infection and which remained in the patient's body. Immunity (acquired) resulted from the destruction of the infecting agent by the enzyme when it tried to cause subsequent infections. Natural immunity could not be explained in this manner.

Vaughan stated that the parasite in this case could not grow in the body tissues. In reality Vaughan's explanation is not very different from some of the others; it is just as reasonable.

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CHAPTER XXXII

VARIETIES OF IMMUNITY

There are several varieties of immunity which may be distinguished from one another. These may be classified as follows somewhat after a method of Muir and Ritchie.

METHODS AND VARIETIES OF ARTIFICIAL IMMUNITY—ACTIVE IMMUNITY ¹

1. Natural Immunity
2. Artificial Immunity (Acquired)
 - A. Active Immunity—i.e., produced in an animal by injection, or by a series of injections, of non-lethal doses of an organism or its toxins.
 1. Attack of the disease
 2. By injection of living organisms.
 - (a) Attenuated in different ways
 - (1) Growing in presence of oxygen
 - (2) Animal passage
 - (3) Abnormal temperatures
 - (4) Weak antiseptics
 - (5) Drying
 - (6) Other methods
 - (b) Virulent—non-lethal doses.
 3. By injection of dead organisms (Bacterins)
 4. By injection of dead organisms sensitized by an anti-serum
 5. By injection of filtered bacterial cultures, i.e., toxins; or of substances derived from such filtrates
- B. Passive immunity—i.e., produced in one animal by the injection of the serum of another animal immunized by the methods of A
 1. By antitoxic serum, i.e., the serum of an animal highly immunized against a particular toxin
 2. By antibacterial serum, i.e., the serum of an animal highly immunized against a particular bacterium in the living and virulent condition.

¹Adapted from Muir and Ritchie's Manual of Bacteriology.

NATURAL IMMUNITY

Natural immunity may be defined as the immunity which an individual possesses without artificial inoculation or without having had the disease. Kolmer defined it as follows:

Natural immunity is the resistance to infection normally possessed, usually as the result of inheritance, by certain individuals or species under natural conditions.

Natural immunity is usually possessed by most of the members of a species. What is regarded as natural immunity to-day may be immunity which has been acquired by the species over a long period of time. We may assume that when a race of people lives in a country where an infectious disease is endemic, the susceptible members of the race will take the disease and either be killed off or survive. Those who survive will possess an acquired immunity. When this process is repeated over and over again through centuries of time, acquired immunity may slowly change to natural immunity. This may be the case with those peoples living in Central America who are now said to be naturally immune to yellow fever. Negroes are known to be much more resistant to yellow fever than white people.

Dr. Vaughan in his "Treatise on Epidemiology" reported the introduction of measles into the Fiji Islands in 1875. The disease had been unknown in these Islands; among a population estimated to be 150,000, there were 40,000 deaths. Vaughan did not explain this high mortality as due to the introduction of a new disease to a people who had had no opportunity to acquire an immunity but rather to the fact that during a widespread epidemic so many are ill that the sick did not have proper care. While this may be the case as far as the deaths are concerned, it does not satisfactorily explain the case rate. This may have been due to the fact that measles was a new disease to these people and that they had had no opportunity to acquire even a slight immunity.

Immunity in Utero.—This subject may be discussed in connection with active and passive immunity. The fetus may become immune by having an attack of the disease in the uterus, from which the mother suffers. This would bestow an active immunity. On the other hand the fetus may not become infected with the disease afflicting the mother but receive immune bodies from the mother through the placental circulation. This would give passive immunity.

Hereditary transmission of infections is determined by many factors such as the character of the maternal infection, the susceptibility of the fetal organism, the disease, etc. One investigator injected blood from

the umbilical cord of a tuberculous mother into animals and secured evidence of the presence of tubercle bacilli in five out of twenty-one cases. This would indicate that there was an interchange of antigen between mother and fetus. It has been proposed that the colostrum might also contribute immune bodies to the infant. Experimental evidence does not support this theory, however.

Active and Passive Immunity.—Two types of acquired immunity are distinguishable, active and passive. They are definitive terms which nicely cover the facts. By *active* immunity is meant one that results from the active participation of the body cells themselves in the defense of the organism. *Passive* immunity differs from this in that the body cells remain inactive or passive. They are not stimulated to form immune bodies of any immediate consequence. In active immunity the protective substances, or immune bodies, are made by the body cells of the patient or subject of infection; in passive immunity they are injected into the patient after they have been made in some other animal. The establishment of active immunity requires time (perhaps several weeks) and therefore cannot be used where it is necessary to instigate the initiation of immunity within a short time. Passive immunity methods are used, then, especially for curing disease; whereas active immunity is used for preventing infection. The active participation of the patient's body cells for the building of active immunity is secured by injection of an antigen. These various agents and methods have been tabulated in another place.

Active and passive immunity may be contrasted in the following manner:

Active Immunity	Passive Immunity
1. The body cells of the patient are activated to form immune bodies.	1. The immune bodies are injected into the patient.
2. Active immunity requires the administration of an antigen with which to stimulate formation of immune bodies. Is produced by an antigen.	2. No antigen required; is produced by an antibody.
3. Time is required for the appearance of immune bodies.	3. No time is required since the immune bodies are injected.
4. Bacterins (bacterial vaccines) are used.	4. Antitoxins are used for bestowing passive immunity.
5. Active immunity methods are preventive or prophylactic.	5. Passive immunity methods are curative methods.

METHODS OF PRODUCING ARTIFICIAL (ACQUIRED) IMMUNITY

The different methods that may be used for bestowing artificial immunity to disease on an individual, are quite diverse. We will now proceed to discuss the important ones. These are used to increase and add to what little immunity one possesses naturally. Natural immunity might not be sufficiently high to protect a person if he received a very large dose of microorganisms. Consequently it is unsafe to rely on natural immunity for protection against infection. These are augmented by the so-called subjective methods for the prevention of disease. They are more direct than the objective methods, such as disinfection, sterilization, pasteurization of milk, etc., but when employed in addition to the latter, give a person considerable assurance of freedom from disease.

An Attack of Disease.—General statements cannot be made here since conclusions are influenced by the disease in question as well as other factors. Perhaps, in a few diseases there is satisfactory immunity following infection. Such may be the case in measles as indicated by the following illustration. Vaughan mentioned the experience of the inhabitants of the Faroe Islands with measles. He stated that in 1781 measles disappeared from these islands and did not reappear until 1846. During this interim of sixty-five years there was not a single case. Finally in 1846 the disease was introduced but none of those who had the disease sixty-five years before suffered attacks. This case would seem to indicate a permanent resistance to measles. A few other diseases are in the same category.

An attack of communicable disease presupposes the presence, in the body of the patient, of the microorganism causing it. Bacteria which have gained entrance naturally act as antigens to cause the production of antibodies (immune bodies) in the same manner probably as do dead bacteria (bacterins) which are administered for the production of artificial (active) immunity. With some diseases, one attack has led to a small number of recurrent cases. Consequently, the belief is generally held that one may not have the disease more than once. Such a statement appears in some texts but it is not in accord with the facts. Recurrent cases of disease are not uncommon in the same individual. It should be stated again that immunity is relative and not absolute. Massive infection, for instance, may overcome a grade of immunity acquired by an attack of the disease or by preventive inoculation. In a few diseases, however, it is known that an attack causes such a high grade of immunity that recurrent cases are uncommon.

While it is possible to have some diseases more than once, it is only

proper to point out that such cases may be explained by incorrect diagnoses. It is possible for an individual to have diseases with symptoms very much alike. An individual may have malaria, for instance, with the diagnosis of typhoid fever. Confusion is especially possible when an agglutination reaction is not made with the patient's blood. Or, the patient may have one of the paratyphoid fevers which have many symptoms in common with typhoid fever, although they are not so severe.

Use of Attenuated Microorganisms.—These are microorganisms which have been devitalized (attenuated) in some manner. They have not been killed but have lost their ability to multiply or, in this case, their ability to cause disease. Such devitalized bacteria have been used for increasing the resistance (immunity) in individuals.

Attenuation by Animal Passage.—This method of attenuating microorganisms was one of the earliest to be used. It is reasonable that the injection of a microorganism into a host which is not normal or which is unsuitable would greatly alter it. The propagation of the smallpox virus in the heifer causes an attenuation or reduction in virulence. Pasteur showed that the virus of rabies could be increased in activity by passage through an animal. This indicates that animal passage will increase as well as decrease the vitality.

One of the most interesting cases of the use of an organism attenuated by animal passage was Friedmann's cure for tuberculosis, an announcement heralded far and wide in lay literature and discontinuously over a number of years in German medical journals. Friedmann proposed the use of a strain of the tuberculosis organism which had been attenuated by passage through a cold-blooded animal. The animal used was the turtle and the altered strain became known as the turtle bacillus. The United States Public Health Service invited Friedmann to come to America to demonstrate his treatment or cure. He accepted but the demonstrations were not successful enough to warrant accepting Friedmann's conclusions.² The attenuation of the tuberculosis organism as Friedmann produced it, was not sufficient to allow the altered organism to be used as a curative agent.

Immunity Methods in Smallpox.—This is one of the oldest known methods of acquiring immunity. Centuries before the Christian era the Chinese people observed the immunity for disease which followed an attack of smallpox. Consequently, we are not surprised that they attempted to confer immunity by artificial inoculation. They did this by rubbing some of the smallpox virus into the skin or by placing the

² The results of these demonstrations are reviewed in Hygienic Laboratory Bull. No. 99, United States Public Health Service, Washington, D. C.

scabs in the nostrils. Such practices caused mild cases of smallpox which left the individual immune; it was found, however, that the disease could be spread as easily from these cases as from those which were naturally infected. The history of smallpox has been briefly touched upon in the first chapter. The preparation of the vaccine virus will be described now.

Preparation of Smallpox Vaccine Virus.—The vaccine virus is a living agent which must be propagated in or on some living animal. In



FIG. 134.—Removing Smallpox Vaccine. (Courtesy Parke, Davis & Co.)

Eight days after a vaccine heifer has been inoculated with "seed virus" the heifer is killed and, after prolonged irrigation of the vaccinated area, the vaccine pulp is collected and transferred to sterilized containers.

former times, it was secured from the arm of a person who had just undergone a successful vaccination. This method was used almost exclusively; it has been said that the people of Richmond, Virginia, were asked to be vaccinated during the Civil War in order that a supply of the virus be available for the soldiers of the Confederate army. The objections to such a practice are apparent. In some cases diseases were handed on from the person from whom the virus was taken to the person on whom it was desired to bestow immunity. It soon became necessary to devise other methods, which are now used, for the preparation of a safe, satisfactory vaccine virus. Healthy heifers are now used on which to grow the vaccine virus. They are held in a receiving stable until qualified veterinarians pronounce them free from diseases to which the heifer

is subject; during this detention period they are also subjected to laboratory tests and other examinations which either cause them to be rejected or admitted to the next steps in the production of vaccine virus. If they are accepted as healthy in every respect, they are shaved over the abdomen and thoroughly washed and sterilized. They are then taken to the operating room where they are prepared for inoculation. The animal is placed on the operating table in such a manner that the areas on the ventral portion of the body which are usually used, are exposed for vaccination. The operators then make linear incisions taking care that blood is not drawn. Glycerinated vaccine virus is then rubbed into these areas; this material is spoken of as "seed virus." The heifer is then kept under strictly sanitary conditions while the virus grows. The stables are kept strictly sanitary by attendants who are constantly on duty. At the end of this period during which the vaccine virus has grown, the lines are found to be covered with small white vesicles which contain the virus. During their growth, all scabs are cleared away as they form in order that none may be collected with the virus. When the virus has grown sufficiently the animal is etherized, placed on an operating table and the "vaccine pulp" scraped off and transferred to sterile containers. This vaccine pulp is then taken to another room where it is thoroughly mixed and treated with glycerol and sterilized distilled water. The material is ground and thoroughly mixed after which it is stored before it is made into the preparations for the market.

Smallpox virus is examined just as carefully, before marketing, as are the other biological products. These tests include laboratory examination for the presence of aerobic and anaerobic bacteria and animal inoculations to reveal the presence of any undesirable bacteria in the preparation. The animal which has been used for the preparation of the vaccine virus is carefully posted by veterinarians after the vaccine pulp has been removed. In this manner the manufacturers do everything possible to make certain that the material comes from healthy animals. They examine the product as well as the animal from which it was collected.

Duration of Immunity Following Smallpox Vaccine Inoculation.—A teacher frequently hears the question, "How long is a person immune after preventive inoculation for smallpox?" Jenner stated that a person was immune for life after successful inoculation; we know this to be untrue and to-day more guarded statements are made. This matter has been recently discussed by Gillihan,³ whose article should be consulted

³Gillihan, A. F. Duration of Immunity Following Modern Smallpox Vaccine Inoculation. Amer. Jour. Pub. Health 17 (1927), 906-911.

by those who desire a longer discussion. Experience to-day seems to indicate that several successful inoculations at different age periods such as during the first year of life, at about the age of seven and finally during adolescence, will protect the subject for life. In Germany two successful inoculations are required by law, one before the child has reached the age of one year and the second before the age of twelve. This practice seems to have stamped out the disease in Germany. Physicians have to go great distances to even see a case of smallpox and many of them never see one. Unfortunately this is not the situation in America.

Reasons for Failure in Attempted Smallpox Inoculation.—Various reasons have been advanced to explain why a person sometimes cannot secure a successful inoculation or why it may require many attempts before one is secured. Deteriorated vaccine virus may be one reason; faulty technic on the part of the practitioner may be another. Some individuals may be naturally immune. Gillihan described the immune reaction which practitioners should look for before stating that the subject is naturally immune or has acquired sufficient immunity to prohibit successful inoculation. Gillihan quoted a report that in the Orient, on account of the virulent type of the disease, reinoculations are made in immunized persons until no reaction at all is secured to the virus. It is also quite probable that some people never reach such a state of resistance. The author just quoted reported a case of a physician in a vaccine plant in England who had to give up this occupation because his hands frequently became inoculated with the virus and the inoculation (vaccination) ran its normal course each time. We must distinguish, of course, between immunity to vaccine virus and immunity to smallpox.

Attenuation by Abnormal Temperature.—This method was used by Pasteur for the preparation of anthrax vaccine. *Bacillus anthracis* was incubated at 43° C., instead of 37° C. A culture of the organism, which originally killed 100 per cent of sheep inoculated with it did not kill any after incubation at 43° C. for ten or twelve days.

Attenuation by Drying—Pasteur Treatment for Rabies.—This method is best known in connection with the preparation of the agent used in the so-called Pasteur treatment of rabies. Pasteur used two terms for the viruses which he prepared. The virus taken from a naturally infected dog was called "street virus;" that which had been exalted in virulence by passage through susceptible animals was called "fixed virus." We have to use the general term virus, for the etiologic agent in rabies is not known. The Pasteur treatment involves the use of this fixed virus. The material to be used in the inoculation to prevent rabies is made as follows:

Rabbits are inoculated with a fixed virus which has been taken from

another rabbit. Just before the inoculated rabbit should die (about the eighth or ninth day) it is killed and its spinal cord is removed aseptically. This cord is then cut into short pieces which are suspended over potassium hydroxide under aseptic conditions. Pasteur found that the storage of spinal cord under these conditions caused it to become so dry that it was no longer infectious. Consequently, he started the prophylactic treatment with an emulsion of spinal cord which had been dried for fourteen days. About 0.5 cc. of the cord is ground up in sterile physiological sodium chloride solution. This constitutes a dose. Subsequent doses are 0.5 cc. of the cord dried for decreasing periods. Towards the end of the treatment, after eighteen days, the patient receives spinal cord which has been dried for only three days. In this manner a person who has been bitten by a dog believed to be rabid may slowly have his immunity raised. The material with which this has been done has been of increasing virulence.

Whether a person needs the Pasteur treatment or not depends on the circumstances which seem to justify its use. The situation is often this. A person may be bitten by a rabid animal or one which showed symptoms of rabies. If there were abrasions in the skin the problem is much worse. The situation is often made much more complex by the shooting of the suspected animal. In such a case the subject has the alternative of taking the Pasteur treatment or taking the risk that the animal was not infected with rabies. Health authorities are united that the suspected animal should not be shot but kept for observation by qualified persons. If the animal later shows no symptoms, the Pasteur treatment need not be taken by those who may have been bitten. If the dog is killed the head should be sent to a reputable laboratory for examination. The laboratory worker examines the brain of the animal for Negré bodies which have been found to be present when the animal is infected.

The history of a case of rabies is so well portrayed by a newspaper report published in 1927 that pertinent portions are given here

Compassion for a homeless dog shivering outside his police booth in Brooklyn cost Policeman ———— his life. . . . The policeman was bitten by the mongrel and considered the wound so inconsequential that he disregarded it. ———— was on duty when the dog approached, dragging an injured leg. The mercury registered six above zero and the dog whined from cold and hunger. ———— opened the door of his police booth into which the animal crept. ———— entered and as he started to stroke the dog it snapped savagely at its benefactor. The policeman sprang back but the mongrel's teeth cut through the cloth of his uniform and nipped him near his groin. The slight scratch seemed so trifling that he said nothing and soon forgot the incident. Two months to a day after he had been bitten, he first felt pain and began to develop symptoms of rabies. He showed extreme hyper-

excitability and was unable to swallow. Five days later he was taken to the hospital where he died after continued convulsions. An examination of the brain showed the presence of Negré bodies.

This is a typical case of rabies. The incubation period may be of varying lengths of time. Some authors have reported periods of incubation of several years.

An attenuated strain of *Mycobacterium tuberculosis* has also been prepared by drying. The cells are dried in sealed tubes and then used as a vaccine or bacterin. Black leg vaccine is also prepared by drying it above 85° C. for seven hours.

Attention by Weak Antiseptics.—Weak antiseptic also have the ability to weaken organisms. Chamberland and Roux reported that *Bacillus anthracis* could be attenuated by growing it in media containing 1 part of carbolic acid per 600 parts of medium. Another more recent example of attenuation with weak antiseptics might be Calmette and Guerin's work with *Mycobacterium tuberculosis*. They have reported striking results in immunity to tuberculosis with a bovine strain of the tuberculosis organism isolated in 1908. They passed the organism through 230 successive inoculations on a medium composed of potato saturated with concentrated bile containing 5 per cent of glycerol. This organism was said to be non-infectious for calves even when large inoculums were given. We need not burden ourselves with a more detailed discussion of their results since their work is of too recent origin to accept as established facts. It does, however, justify the attention of more advanced students of bacteriology and immunology. The bacterin prepared by Calmette and Guerin is known under the designation BCG (*Bacillus Calmette Guerin*).

Besides the various chemical reagents which have been used for attenuating microorganisms, light has also been used. In searching for methods for attenuating the tuberculosis organism V. and Mme. Henri used ultra-violet light. The results have not been satisfactory. It is definitely established that ultra-violet light destroys bacteria; one may wonder why it may not be used for attenuating them.

Use of Non-lethal Doses of Living Bacteria.—This method, while theoretically possible, would be a difficult one to control with many microorganisms. It is very difficult to determine how many bacterial cells are necessary to cause disease. Some reports state that as few as one cell will cause infection. The determining factors probably are the resistance of the host and the aggressive powers of the microorganism. It is possible that one cell of a virulent organism might be given to a subject with low resistance and cause a serious infection. The safe method, if living organisms are to be used, is to employ an organism which has

been attenuated in some manner. Living bacteria may be responsible for immunity of many of us to tuberculosis. Surgeons who perform great numbers of autopsies have reported evidence that practically everyone is, at one time or another, infected with tuberculosis. In many cases the lesions have been sealed off by nature. The living cells apparently stimulated the defensive powers of the host to protect.

Active Immunity by Injection of Dead Bacteria (Bacterins).—Dead bacterial cells may be used for bestowing active immunity. The cells must be destroyed in a certain manner else their ability to stimulate the body cells of the subject to form immune bodies will be destroyed. These products are known as "bacterins" or bacterial vaccines and are used as agents in active immunity. The bacterial cells to be used as bacterins are usually killed by heating at 60° C. for one hour. The temperature is kept at this point because a higher temperature will destroy the antigenic properties of the bacterin. Bacterins, or bacterial vaccines, cannot be used for conferring passive immunity.

Preparation of Bacterins of Bacterial Vaccines.—The bacterial cells used in the preparation of bacterins are grown in pure culture on the surface of solid media such as plain agar. The medium is usually contained in broad, shallow flasks in order that there be as much surface area as possible. After growth has occurred, the cells are washed and scraped off in sterile physiological sodium chloride solution. This suspension is then thoroughly shaken to break up the clumps and yield an even suspension. About 0.1 per cent of tricresol is added to the suspension, which is counted and diluted. Two suspensions are generally made; that for the first injection contains 500,000,000 cells per cubic centimeter; the two subsequent injections 1,000,000,000 cells per cubic centimeter. The lowest concentration constitutes the first injection while the heavier suspension is used for the second and third injection. It is necessary to distribute the injections over a period of time since the administration of all of the cells (2,500,000,000) at once would cause too great a reaction.

Confusion frequently exists in students' minds on bacterins and antitoxins. In the following these products are contrasted:

Bacterins	Antitoxins
1. Used in active immunity	1. Used in passive immunity
2. Prepared from bacterial cells; usually killed by heat	2. Prepared in the body of some animal; usually a horse
3. Used to prevent disease	3. Usually used for curing disease
4. Used in diseases in which the etiologic agents form intracellular toxins	4. Used in diseases in which etiologic agents form extracellular toxins

Stock Bacterins and Autogenous Bacterins.—Two types of bacterins or bacterial vaccines are used; one is called a stock bacterin and the other an autogenous bacterin. A stock bacterin is one prepared in large quantities by manufacturers and kept in stock at depositories. These bacterins are prepared in the manner outlined above. In order to increase the value of such bacterins, manufacturers have prepared stock bacterins from a number of cultures or strains of the same microorganism. This yields what is known as a *polyvalent bacterin*.

An autogenous bacterin is one prepared from the organism causing the infection. It is reasonable to expect that a bacterin prepared from the organism which causes an infection to be more specific than a stock bacterin. The organism is isolated from some excretion and propagated in pure culture and a bacterin prepared in the same manner as the stock bacterins.

The relative advantages and disadvantages of stock and autogenous bacterins are apparent from the above discussion. Stock bacterins are less expensive and are available in a short time. Autogenous bacterins must be prepared from material taken from the subject to whom they are to be administered and from three to five days are usually required to make an autogenous bacterin and test it for purity and sterility. Any objection that can be brought against the autogenous bacterins is negated by the one that they are more specific and give the best results.

The bacterins, or bacterial vaccines, mentioned above are known as saline bacterins because the cells were suspended in physiological sodium chloride solution. The best practice for administering saline bacterins is to give three injections as outlined above. In times of peace or when there is no emergency this method may be followed but in time of war or other great emergency it would be more satisfactory to be able to immunize with one injection. Consequently, in order to be able to use one injection and to avoid the reaction which usually follows when a saline bacterin is administered, during the War the cells were suspended in oil (olive, cottonseed, etc.), to give what was known as *lipobacterins*. The use of lipobacterins, however, was discontinued after the War. The comparative studies which have been made fortunately indicate that the saline bacterins are superior to the lipobacterins.

Results of Prophylactic Inoculation.—Statistical data are often neglected by students. The following should interest even the most disinterested. It deals with one of the most striking contributions of science to human welfare. A prominent state board of health weekly bulletin states that prior to 1910 when the use of antityphoid bacterin was first begun in the United States Army, from 20 to 100 soldiers in every 10,000 had typhoid fever. By 1912 when protective vaccination

had come into more general use, the typhoid rate had been reduced to three in every 10,000 and in 1915 only 8 of every 100,000 soldiers had typhoid. Surgeon Ireland of the United States Army was quoted as follows:

The World War demonstrated, on the most vast scale possible, the very great value of prophylactic typhoid vaccine as a preventive measure. During the Spanish-American War about 20,000 soldiers suffered attacks of typhoid and about 2000 died of it, whereas during the World War there were only about 2000 cases with approximately 200 deaths. These gross figures, striking though they be, fall far short of representing the actual results obtained in the prevention of typhoid. The number of men mobilized for the Spanish-American War was only about 250,000 while during the World War approximately 4,000,000 men were called to active service. Considered, therefore, in the light of the actual strength of the American armies engaged in the two wars, it can be stated that for every *one* case of typhoid occurring during the World War 150 occurred during the Spanish-American War.

Our armies fared much better in this respect than did the armies of our Allies. The mobilization of the French and British armies was of necessity a very hurried one and the existing emergency prevented the widespread use of this prophylactic measure. In the French armies, for example, 45,000 cases of typhoid and paratyphoid occurred during the first six months of hostilities. Not until about the end of the second year of the War was it possible for the French to apply very completely this prophylactic measure. The value of its use is evidenced by the fact that during the first two years of the war, when vaccine was administered only to limited numbers of men, approximately 100,000 French soldiers had typhoid and paratyphoid fevers, whereas during the last two years, when it was possible to apply this measure generally, only about 2000 cases occurred. The armies of all of our Allies had a somewhat similar experience, except that the British succeeded in initiating this prophylactic measure through every part of their forces within a few months of the outbreak of the War, with the result that this group of diseases was immediately brought under control.

To-day cases of typhoid fever are looked upon as medical curiosities in Army hospitals. During 1925, with an average strength of 136,000 men, only 4 cases of typhoid occurred in the Army. One of these had recently been enlisted and was not protected by vaccination. The diagnosis was confirmed by recovery of the typhoid organism from the blood. The remaining three had been vaccinated previously. The diagnosis in these cases was based on clinical symptoms and could not be confirmed by laboratory examinations, though such examinations were made. All patients recovered.

Period of Immunity.—How Long Does Immunity Last after Preventive Inoculation? This question cannot be answered directly because there are many factors which influence the answer. In the first

place, there are probably no satisfactory methods for measuring immunity in terms of units that could be used. Individuals vary greatly in their response to preventive inoculation. Usually, soon after the first two injections of typhoid bacterin, immunity to typhoid fever is manifest. The opinions of army officers are quite reliable in this connection. They inoculate so many individuals under controlled conditions that they have a better opportunity to study this question than many civilian medical officers of health. Army officers believe that immunity to typhoid fever begins to decline after from two to two and a half years, but may last longer since the incidence of typhoid fever among inoculated troops is low even after four or five years.

Reasons for Failure of Preventive Inoculation.—We should again stress the fact that immunity is but a relative matter and may not always prevent infection. Numerous reports are available in medical literature, especially that of the war period, dealing with outbreaks of typhoid fever among those who had been inoculated. These outbreaks should not be used as arguments against preventive inoculation, since there are satisfactory explanations. Some of them will be discussed.

Low-grade Bacterin.—If the bacterin has not been properly prepared, it cannot function as a good antigen or agent with which to stimulate the formation of immune bodies. Bacterial cells which are to be used as bacterins should not be killed above a temperature of from 63° to 65° C.; the temperature is usually kept below 60° C. They should also be stored in a cool place although it is known that this is not an important factor over a short time.

Mistaken Diagnosis.—Another possible reason for failure is giving the wrong bacterin. There are some diseases which are very much alike. Typhoid fever and the paratyphoid fevers have symptoms in common. A person may receive prophylactic inoculation against typhoid fever, for instance, and later have paratyphoid fever or even malaria; the diagnosis may be typhoid fever.

Massive Infection.—It has been mentioned before that immunity should be regarded as a relative matter and not an absolute means of disease prevention. Every one possesses a certain amount of natural resistance. This may be raised by inoculation to a level whereby there is no susceptibility to ordinary doses of bacteria. Massive doses, however, frequently overcome such immunity causing cases of disease. This explanation was given during the War for cases of typhoid fever among inoculated troops.

Lowered Resistance.—Fatigue, hunger, and the like, help to lower a person's resistance to infection. They may so lower it that the immunity received from inoculation is overcome.

Sensitized Bacterins.—These are known as serobacterins or sensitized bacterial vaccines. They are made by mixing the bacterin with immune serum, allowing the mixture to stand for twenty-four hours and then washing the cells with physiological sodium chloride solution. The bacterial cells are then suspended in salt solution, standardized and put up in packages for distribution. Such cells are said to be sensitized because they have absorbed from the serum certain agents called *amboceptors* which cause them to be more easily destroyed by the body cells of the individual. Their use is somewhat limited.

Active Immunity by the Use of Bacterial Extracts.—This is a broad topic which covers a number of products prepared from bacterial cells. These materials were introduced because it was believed that the immunizing portion of the cells could be secured for use. These attempts have not yielded much success. The *tuberculins* are, perhaps, the best examples of such preparations.

Tuberculin.—In 1890 Robert Koch announced the discovery of a substance in cultures of *Mycobacterium tuberculosis*, which caused a specific reaction in those who were afflicted with tuberculosis. He further stated that this substance, if administered over a sufficiently long time, would cure the disease. Would that further experimental work had confirmed this. The next year Koch's work was repeated by Bujwid who proposed the name *tuberculin* for this agent. This name was adopted by Koch. Immediately after Koch announced the curative properties of tuberculin, it was generally administered but without the success reported by Koch.

Several different types of tuberculin are known. The first one, now spoken of as "old tuberculin" to distinguish it from tuberculin prepared by modified methods, was prepared from pure cultures of *Mycobacterium tuberculosis*. The organism was grown for five or six weeks on a 5 per cent glycerol broth. After this time the culture was concentrated to about one-tenth of its original volume and filtered through sterile filters to remove bacteria. This "old tuberculin" contained, then, besides glycerol, the substances which were in the cells of *Mycobacterium tuberculosis*, the products of metabolism, undecomposed medium, etc.

Use of Tuberculin in Diagnosis.⁴—Tuberculin has enjoyed some use as a diagnostic agent. It may give indication of the presence of tuberculosis in individuals where other methods are unsatisfactory. When tuberculin, in comparatively large amounts, is injected into uninfected individuals, no symptoms result. When, however, it is injected into an

⁴ Tuberculin. A Report of a Conference on its Standardization. Supplement Public Health Reports No. 57 (1926). U. S. Public Health Service.

individual suffering from this disease, there is a feeling of malaise with headache, backache and a distinct rise in temperature. A local reaction is also secured at the site of the injection. It is used, especially, to detect tuberculosis in cattle. Animals which give a rise in temperature after injection with tuberculin are said to be "reactors" and are believed to be infected. The objections to the test must be left for other texts.

Von Pirquet also used tuberculin in a skin test to show the presence of tuberculosis infection. A little tuberculin is applied to a scarified area of the skin; a positive test is indicated by a reddened area varying from a local hyperemia to an inflamed area. Another test, the Calmette test, involved the addition of a little diluted tuberculin to the eye of suspects; the appearance of a congested condition indicates the presence of tuberculosis.

METHODS OF PASSIVE (ACQUIRED) IMMUNITY

The methods of passive immunity, as indicated before, rest on the administration of antibodies, or immune bodies, which have been made in another animal. The body cells in the human being to whom the immune bodies are administered remain passive and are not concerned with the immunity which is immediately established. They are not stimulated to form their own immune bodies. Such immunity is also passive in the sense that it is not as permanent as active immunity. It disappears more quickly.

Two types of antisera are used for the bestowal of passive immunity, antibacterial sera and antitoxic sera. These have been discussed before.

REFERENCE

BROADHURST, J. *How We Resist Disease: An Introduction to Immunity*. J. B. Lippincott Co., Philadelphia, 1923.

See Appendix for names of other books which may be consulted.

CHAPTER XXXIII

BACTERIA IN PLANT DISEASES

Plants like other living organisms seem to be susceptible to attack by bacteria and other microorganisms. Just as there are departments and bureaus in government organization for the study of diseases of man and animals, so are there departments and bureaus for the study of diseases. Diseases of plants¹ cause great losses to agriculturists and every attempt is made to fight them. The literature has become voluminous and is reviewed in several books the names of which are listed at the end of this chapter. Only a general statement for the student will be attempted here. The discussion may be organized in different ways; however, a few common diseases of several plants are discussed under the heading of the type of infection which is caused.

Anderson gave the general symptoms of diseases of fruits as follows:

1. *Spots on Leaves or Stems*.—Spots with more or less regular outlines appear on the surfaces of the leaves. Such diseases are called leaf spots. If the central dead area drops out, a shot-hole effect is produced. Examples: leaf spot of cherry and bacterial shot-hole of peach. Similar spots may appear on the stem, as in raspberry anthracnose.

2. *Wilt or Blight*.—The ends of branches, or the entire plant, wilt or die rather suddenly without the leaves falling. Examples: twig blight of pear and apple.

3. *Yellow, Sickly Foliage*.—This may or may not be followed by defoliation or death of the plant or branches involved. A condition of this nature usually indicates some trouble with the root system or crown of the tree.

4. *Defoliation, or Dropping of Leaves*.—Example: cherry leaf spot.

5. *Rusts*.—Yellow or orange-colored spots on leaves, fruit, and twigs, without death of the host tissues, are often caused by rust fungi. Examples: orange rust of blackberries and apple rust.

6. *Mildew*.—A white or grayish growth on the surface of the leaves of young shoots is produced by the mycelia of certain superficial fungi, the *powdery mildews*. There is also a class of *downy mildews* which produce a more dense, grayish growth usually confined to a small area.

7. *Spots, Blotches, or Scab Areas on the Fruit*.

8. *Rots on the Fruit*.—Usually starting as a minute water-soaked area the rot spreads and frequently involves the entire fruit. Rots may be soft or firm, dry or wet, dark or pale, or may vary between these extremes.

¹Stevens, F. L. The Relation of Plant Pathology to Human Welfare. Amer. Jour. Bot., 8 (1921), 315-322.

TABLE X
PLANT DISEASES AND THEIR CAUSE

Name of Disease	Name of Parasite	Date and Discoverer	Type of Disease
<i>Rots</i>			
Black cabbage rot	Phytophthora campestri	1893, Pammel	Withering and yellowing of leaf
Soft rot of sugar beets	Phytophthora beticola	1893, Smith, Brown & Townsend	Rot of the beet
Soft rot of hyacinth	Bacillus hyacinthi septicus	1899, Heinz	Rot of the bulb
Soft rot of carrot	Erwinia carotovora	1901, Jones	Soft rot
Soft rot of muskmelons	Erwinia melonis	1910, Giddings	Soft rot of melon
Bud rot of the coconut	Escherichia coli *	1912, Johnson	Rot of the bud
Rot of cauliflower	Erwinia oleraceae	1904, Harrison	Soft rot of roots
Stem rot of potatoes	Phytophthora phytophthora	1904, Appel	Destructive soft rot of stems
Bud rot of canna	Phytophthora cannae	1921, Bryan	Bacterial bud rot
<i>Wills</i>			
Wilt of sweet corn	Phytophthora stewartii	1897, Stewart	Leaves wilt one at a time
Wilt of tomato	Phytophthora solanaceae	1896, Smith	Plant wilts and become yellow
<i>Leaf Spots</i>			
Citrus canker	Phytophthora citri	1915, Hasse	Spots on leaves
Bacterial spot of peach	Phytophthora pruni	1903, Smith	Small watery spots
Leaf spot of larkspur	Bacillus delphini	1904, Smith	Black spots on leaves
Leaf spot of cotton	Phytophthora malvacearum	1901, Smith	
Leaf rot of tropical orchids	Erwinia cyripedia	1911, Hari	Dirty depressed spots on leaves
Leaf spot of cow peas	Phytophthora vignae	1911, Gardner & Kendrick	Bacterial leaf spot
Leaf spot of lima beans	Phytophthora viridifaciens	1911, Tisdale & Williamson	Bacterial leaf spot
Leaf spot of celery	Phytophthora api	1921, Jagger	Leaf spot
<i>Blights</i>			
Blight of pears and other trees	Erwinia amylovora	1878, Burrill	Blight of blossoms, twigs, etc.
Blight of beans	Phytophthora phaseoli	1897, Smith	Attacks foliage, pods, stems, etc.
Blight of lettuce	Phytophthora viridilivida	1915, Brown	Burned, dried appearance of leaves
Blight of mulberry	Phytophthora mori	1908, Smith	Leaves are attacked and turn black
Blight of tomato	Phytophthora michiganensis	1910, Smith	Wilting and yellowing of leaves
	Pseudomonas avenae	1909, Manns	Yellowing of the leaf
Blight of oats	Bacillus avenae	1916, Sackett	Leaves become dark and finally black
Blight of peas	Phytophthora pisi	1893, Pierce	Infection of the stems
Blight of walnut	Pseudomonas juglandis	1916, Paddock	Stem infection
Blight of alfalfa	Phytophthora medicaginis	1924, McCulloch	Bacterial blight
Blight of gladioli	Phytophthora gummisudans		
<i>Galls</i>			
Crown gall	Phytophthora tumefaciens	1907, Smith	Gall formation near ground line

* There is some evidence to refute this organism as the cause of bud rot of the coconut.

9. *Cankers*.—These “sores” on the trunk or limbs usually result in dead areas. In some cases cankers are in the outer portion of the bark only.

10. *Galls, Tumors, or Abnormal Enlargements*.—Galls on leaves, branches, and stems may be produced by the sting of certain insects. Crown gall of numerous plants, however, is caused by a bacterium.

Lack of space prevents the consideration of all of these diseases. The student who is especially interested will find an interesting discussion and illustrations for all of the above symptoms in Anderson's publication.

BLIGHTS

This is a fitting disease to discuss first since it was the first to be definitely proven to be due to a bacterium. Several common blights are discussed below. The symptoms are given in each instance.

Pear Blight or “Fire Blight.”—This is a serious disease of pear trees which also attacks the apple and quince and occasionally the plum. While the actual loss from this disease is hard to determine, Anderson stated that it was safe to say that it averages yearly 25 per cent of the potential bearing power of the trees in the United States. He stated that frequently 90 per cent of the blossoms in an orchard are blighted while the loss of entire orchards is not uncommon.

Fire blight is apparently an old American plant disease since it is known to have been observed in 1794 in orchards along the Hudson river. It seems to have spread rapidly over the United States and Canada, following quite closely the development of orchards.

The organism causing blight of pear trees was discovered by Burrill at the University of Illinois in 1887. The organism was finally named *Bacillus amylovorus*. (According to the new classification which has, in general, been followed in this book the name is *Erwinia amylovora*. The generic name in this case memorializes one of America's great plant pathologists whose death occurred in 1927, Erwin F. Smith of the Bureau of Plant Industry, United States Dept. of Agriculture.) The bacterial parasite lives in the host but does not seem to be resistant to unfavorable conditions. Consequently it does not survive long in dead tissue. During the winter it remains dormant in the tissues of the host. During the spring when the host is again active the bacteria multiply rapidly and many appear in a sticky secretion from the lenticels. This material is then distributed by insects and the disease is spread. Undoubtedly, other methods of dissemination exist but the one just mentioned is an important natural one.

Blight of the pear is characterized by a blackening of the leaves and also by a blackening of the bark on young twigs. On account of the

fact that the leaf or bark appears burned, the disease has been also called fire blight. All varieties of the pear are susceptible. The blossoms may also be attacked and readily show symptoms. Better evidence of blight, however, is observed on the twigs.



FIG. 135.—Twig Blight of the Pear Showing Exuding Drop at *a*. (After Anderson, 1920.)

Anderson reported no successful methods for the control of this disease. He mentioned the following points regarding the nature of the disease as related to attempts to control it:

- (1) It is caused by a bacterium, not by a fungus.
- (2) It is usually introduced into the flower or twig in such a manner as to pre-

clude the possibility of applying a spray for protective purposes, i.e., of meeting the organism before it enters and preventing its multiplication.

(3) After the bacteria enter the tissues it is useless to attempt to kill them by external applications of sprays.

(4) The disease is usually, but not always, insect-borne.

(5) The bacteria may be transferred by wind-blown mist.

(6) Infection on twigs most frequently occurs through the puncture of insects.

(7) When first introduced into the blossoms the bacteria multiply rapidly in the nectar of the flowers.

(8) High temperatures and dry weather are highly unfavorable for the development and spread of the disease.

Blight of Lettuce.—This disease was found in the southern part of the United States where about 200 acres of lettuce plants were infected. The fields looked as if a fire had swept through them. The disease was evidenced by a shriveling of the outer leaves of the head with some of them in a soft rotted condition. The centers of the heads examined by Brown² were sound but between this sound area and the outer dead leaves were others affected in varying degrees. In some places numerous spots appeared with a water-soaked appearance. Agar plates were poured and the etiologic agent was isolated. The organism was proved to be infectious. Brown suggested the name *Bacterium viridilividum*. According to the newer classifications this name becomes *Phytomonas viridilivida*.

Blade Blight of Oats.—This disease is evident when the oat plants are from 6 inches to a foot high. The leaves suddenly turn brown and die at the tops. The lower leaves are attacked first and the brown color soon extends their entire length. This condition soon extends itself but finally the oat plant may seem to revive, put out new leaves and mature. The crop, however, is materially reduced, the loss reaching as high as 75 per cent in some cases. Manns³ stated that the disease was not restricted to oats, similar troubles having been reported upon wheat, grasses, alfalfa and other crops.

The etiologic agent has been found to be a symbiosis between two bacteria named *Pseudomonas avenae*, a white organism, and *Bacillus avenae*, a yellow organism. Manns stated that the virulence and activity of the white organism depended very much on the presence of the yellow species. The disease could be reproduced by artificial inoculation. Stomatic infection was the chief method by which the plants were afflicted. Bruised plants took the infection much more easily and

² Brown, N. A. A Bacterial Disease of Lettuce. Jour. Agr. Res., 4 (1915), 475-478.

³ Manns, P. F. The Blade Blight of Oats, A Bacterial Disease. Ohio Agr. Exp. Sta., Bull. 210, 1909.

quickly than healthy plants. Insects may also aid in the dissemination of the bacteria. Other details of the disease together with illustrations, some of which are in color, may be found in the bulletin by Manns.

Bacterial Blight of Beans.—The bacterial blight of beans by an organism named *Phytophthora phaseoli* (*Pseudomonas phaseoli*) infects the cotyledons, stalks, pods, seeds, and leaves. Gloyer⁴ stated that lesions on the stalk or pod may or may not be associated with leaf blight. The organism is disseminated on the seed. The organism may apparently infect the bean plant without the formation of surface lesions. The cells of the parasite may appear in the cotyledons from infected seed and pass down into the stem of the young plant. This systemic infection is of importance in seed selection and may explain the epidemics which appear late in the summer.⁵

LEAF SPOTS

As the name indicates, the lesions of disease appear on the leaves. A few of the more common or serious diseases of this nature will be discussed.

Citrus Canker.—This is a serious disease of citrus fruits, study of which has resulted in its control. Stevens⁶ believed it to be the worst citrus disease that was introduced into Florida but that eradication efforts have about rid the state of it. Citrus canker attacks all varieties all citrus trees, except one, the kumquat. Any part of the tree above the ground may become infected. The distinguishing feature of citrus canker is the characteristic spotting of the fruit and foliage. The infection usually appears as small, light brown spots from less than one-sixteenth to one-quarter of an inch in diameter. The spots are usually round and may occur either singly or run together forming irregular areas. The spots are raised above the surrounding healthy tissue and are composed of a spongy mass of dead cells covered with a thin white or grayish membrane. On the leaves lesions first appear as small watery dots with raised convex surfaces. In some cases the spots appear on both sides of the leaf. The spots may also appear on the twigs and fruit.

⁴ Gloyer, W. O. Bacterial Blight of Beans under Field Conditions. Absts. Bact., 6 (1922), Abst. No. 109.

⁵ Burkholder, W. H. The Bacterial Blight of Bean: A Systemic Disease. Phytopath., 11 (1921), 61–69.

⁶ Stevens, H. E. Florida Citrus Diseases. University of Florida Agr. Exp. Sta., Bull. 150, 1918.

Citrus canker is a bacterial disease caused by an organism called *Phytophthora citri* (*Pseudomonas citri*). The parasite is found in great numbers in the spots or lesions. When these spots become moist from rain or dew the bacteria are exuded in masses. These cells may then be disseminated and when they come in contact with the tender tissues of twigs and fruit, new canker spots are formed.

The disease is fought by a complete destruction of all infected trees and a strict quarantine against the introduction of suspected stock.



FIG. 136.—Citrus Canker Spots on Leaves and Stem. (After Stevens, 1918.)

Bacterial Leafspot of Celery.—This disease⁷ is confined to celery in New York and Michigan. The cause of the disease has been named *Phytophthora apii* (*Pseudomonas apii*). The spots are of a rusty brown color, irregular in outline. The characteristic spots have been reproduced by inoculating healthy plants with the organism.

Bacterial Spot of Tomato.—This is a typical plant disease⁸ affecting the fruit, stem and leaf. It occurs on all varieties of tomatoes as well

⁷ Jagger, I. C. Bacterial Leafspot Disease of Celery. Jour. Agr. Res., **21** (1921), 185–188.

⁸ Gardner, M. W., and Kendrick, J. B. Bacterial Spot of Tomato. Jour. Agr. Res., **21** (1921), 123–156.

as on pepper. The organism causing this disease has been named *Phytomonas exitosa* (*Bacterium exitosum*). Foliage infection is stomatal and follows spraying the parasite over the foliage. Fruit infection results from injury; mature fruit, however, is not susceptible probably on account of the high acidity. The organism lives over the winter on the seed.

Bacterial Leafspot of Delphinium.—This disease⁹ is characterized by the formation of tarry, black spots on the leaves, stems, or flower buds. These spots may be 1 cm. across and so numerous as to occupy almost the entire leaf. The bacteria enter the tissue by way of the stomata and water pores. The organism which causes this disease has been isolated and named *Bacterium delphinii*. The organism has not been found to infect other plants. For control it was suggested that all diseased material be burned and that spraying of the soil with Bordeaux mixture be employed.

ROTS

These, as the name indicates, are destructive decomposition of the plant tissue. A great many have been described. But a few will be considered below.

Cabbage Rot.—The name of this disease indicates the major characteristics. It is a very destructive disease of cabbage and cauliflower. The chief diagnostic characteristic of the disease is the appearance of black streaks in the woody portion of the stem and in the leaf stalks. An epidemic of the disease occurred on Long Island in 1895-6. In some cases entire fields were destroyed. The disease usually appears in the latter part of July or when the more advanced plants of late cabbage are beginning to form heads. The first symptoms include the appearance of a wilted condition and a lighter green color. Black streaks are visible in many of the water-carrying fibrovascular bundles. The upward movement of the water carries the disease up into the leaves. When the bundles supplying a portion of the leaf becomes infected, the leaf dies for lack of water.

These symptoms are due to growth in the tissue of an organism *Phytomonas campestris* (*Pseudomonas campestris*). That this is the real etiologic agent is evidenced by the fact that Koch's postulates may be fulfilled with this organism and healthy plants. The organism is apparently able to live in the soil although attempts to isolate it have not been entirely satisfactory.

⁹ Bryan, M. K. Bacterial Leafspot of Delphinium. Jour. Agr. Res., **28** (1924), 261-270.

Stewart and Harding¹⁰ mentioned at least three avenues through which the bacterium gains access to the plant, through wounds, through cuts caused by feeding insects and by infection through water pores.

Satisfactory methods for combating the disease are not known. Certain investigators have advised removing all affected leaves at frequent intervals. Stewart and Harding found this plan worthless for a number of reasons. Such treatment retards the growth of the plant; infection occurs through the roots as well as through the leaves; lastly, infection may occur at the base of the leaf close to the stem and infect the stem.

Cauliflower Rot.—This disease was found to affect the cauliflower, turnips and cabbages in an area about Guelph, Canada. About forty varieties of turnips were tested all of which were more or less susceptible. The disease was characterized by typical rotting and softening, which need not be described in this place in great detail. Harrison¹¹ isolated an organism to which the name *Erwinia oleracea* (*Bacillus oleraceae*) was given, as the etiologic agent. This microorganism reproduced the disease when inoculated onto healthy plants and in this manner Koch's postulates were fulfilled.

Soft Rot of Carrots.—This disease was described by Jones in 1909. The carrot rot is similar to that of other vegetables. The carrots were grown on a field on which carrots had never been grown before so far as could be determined. During harvest an occasional root was found to have rotted but during storage rotting increased. The same situation was experienced the next year. The roots showed a rapidly progressing soft rot which had apparently begun either at the crown or at the root tip, progressing rapidly through the core. The decayed portion was found to be softened and brown in color. This decayed portion was found to be swarming with bacteria and cultures indicated almost a pure culture. Sound carrots inoculated with this organism decayed rapidly. This organism was studied and named *Erwinia carotovora* (*Bacillus carotovorus*). As remedial measures, Jones advised avoiding the use of susceptible plants and contaminated compost or manure. The surfaces of the roots should be allowed to dry thoroughly before storage and benefit would ensue if they were allowed to lie as long as possible exposed to sunlight. The temperature of the storage room should also be kept as low as possible without freezing.

¹⁰ Stewart, F. C., and Harding, H. A., in New York Agr. Exp. Sta., Bull. 232, 1903. See also Wisconsin Agr. Exp. Sta. Bull. 65.

¹¹ Harrison, F. C. A Bacterial Disease of Cauliflower (*Brassica oleracea*) and Allied Plants. Cent. Bakt., Pt. II, 13 (1904), 46-55, 184-198.

Soft Rot of Muskmelon.—This disease¹² was discovered in an experimental field. The season had been dry followed by heavy rains. The melons cracked, furnishing excellent portals for the etiologic agent to enter. The succulent tissue, of course, made a good medium upon which the bacteria could grow. A soft rot resulted beginning on the under side of the fruit and spreading rapidly. The skin became shrunken over the infected areas but was generally unbroken. When the melon collapsed there was a frothing and a disagreeable odor. The organism causing this soft rot was isolated and named *Erwinia melonis* (*Bacillus melonis*). That the organism was the cause of the soft rot seems to have been confirmed by the fact that inoculation experiments reproduced the rot. In this manner Koch's postulates were fulfilled.

WILTS

Sweet Corn Wilt.—This disease was well described by Stewart,¹³ to whom this book is indebted for the following facts. The affected plants wilt and dry up without apparent cause. This generally occurs about the time of flowering. The most distinctive characteristic of the disease is seen when the stem is cut lengthwise. The fibro-vascular bundles appear as yellow streaks in the white parenchyma; in plants which have been dead for some time some of the bundles may be black instead of yellow. When the stem is cut crosswise a yellow exudate appears; this is composed of bacteria. The bacterium causing this disease was not named by Stewart although he proved its etiologic relation to the disease.

GALLS

Galls are large tumor-like growths which appear on certain plants. Large losses have been suffered by nurserymen on account of the disease which, in plants, is probably due to a bacterium.

Crown Gall.—This disease is characterized by the formation of a gall or tumor usually on the scion just above the roots. They vary in size depending on the host as well as the conditions which exist in the several hosts. The disease is also known as "root knot" and "hairy root." This disease occurs on a great variety of plants and is not always on the crown, for any part of the root or shoot may be attacked. Young tissue seems to be more susceptible to attack than older plants. Smith stated that by repeated inoculations through a series of years plants were obtained which seemed to be more resistant to infection. This part of

¹² Giddings, N. J. A Bacterial Soft Rot of Muskmelon Caused by *Bacillus melonis*, n. sp. Vermont Agr. Exp. Sta., Bull. 148, 1910.

¹³ Stewart, F. C. A Bacterial Disease of Sweet Corn. New York Agr. Exp. Sta., Bull. 130, 1897.

the investigations was not satisfactory but suggests the interesting possibility that immune reactions may occur in plants as well as in animals.

A bacterium by the name of *Phytomonas tumefaciens* (*Bacillus tumefaciens*) is known to be the cause of crown gall. Smith and his



FIG. 137.—Showing Crown Gall on A, Apple, and B, Raspberry. (After Anderson, 1920.)

colleagues proved the infectious nature of the microorganism in many inoculations. The parasite has been shown to occur in the primary tumor and also in secondary tumors. It grows well on ordinary media but gradually loses its virulence. The microorganism seems to be able to exist in the soil and attempts should be made to keep the land free from it.

There seems to be no satisfactory method of controlling gall. Anderson stated that cutting off the tumors was not reliable because internal

strands existed which could not be removed. In nurseries, for instance, he advised the avoidance of unnecessary wounding of stems and roots. Instruments used in grafting should be frequently sterilized and wounds caused by grafting should be wrapped. These instructions have much in common with the treatment of animal diseases.

Analogy between Crown Gall of Plants and Animal Cancer.—Evidence of this analogy has been put forward by E. F. Smith, who since



FIG. 138.—Crown Galls on Sugar Beets Two Months After Inoculation with *Bacterium Tumefaciens*. (After Smith, 1923.)

1907 has been an ardent supporter of the theory of resemblance between crown gall and animal cancer. At first this proposal was not accepted by plant pathologists and medical men but to-day quite a few see considerable to support it. The situation is confused somewhat because there are several different explanations as to how cancer and tumors are formed. Smith believed that in crown gall there were "peculiarities of neoplastic growth which remove it from all ordinary plant diseases

and place it in the category of the true tumors (atypical blastomas).” The phenomena of growth in crown gall was said to be surprising and unlike anything hitherto known in plant pathology. Smith believed that for comparative phenomena we must turn to animal pathology, especially to that dealing with malignant tumors. Consequently, Smith in his later writings argued that crown galls were to all intents and purposes cancers.

Animal cancer is now believed to result from irritation perhaps parasitic in origin, perhaps not. The organs which are subject to the irritation are unable to endure it due to acquired or transmitted weakness. Several different parasites have, at various times, been offered as etiological agents in cancer. At present, however, cancer is not looked upon as a parasitic infection.

Control of Plant Diseases.—The various methods of controlling plant diseases are not materially different from those for animal diseases. Anderson enumerated the following practices for dealing with diseases of fruits. These methods may probably be applied to the control of diseases in other plants.

1. *Spraying.* In general, spraying is practiced against fungi which gain entrance to the host through infection by means of a germ tube. It is usually ineffective against bacterial diseases, those caused by adverse environmental conditions, and other so-called physiological diseases. It is also ineffective, as a rule, in the case of canker diseases, where the fungus lives from year to year in the bark or wood. It may aid in the control of these maladies, in that the original infection may be prevented.

Spray mixtures are chemical poisons which are applied to the external surface of the plant in such a manner as to kill the germ tube of the fungus, or prevent the germination of the spore, or, rarely, to kill the superficial mycelium of the fungus. On this account the surface must be thoroughly coated. The spray must be such that it stays on the surface during a fairly long period and must not be easily washed off. It is also essential that it be non-poisonous to the plant to which it is applied. These conditions are met only when the chemicals which poison the fungus are not readily dissolved, yet go into solution in sufficient quantity to prevent the development of the fungus. Frequently the problem is further complicated by the fact that a spray mixture which will not injure one kind of fruit will seriously damage another. Thus, apple trees may be sprayed with solutions which would seriously injure peach trees.

2. *Cutting Out and Destroying Diseased Parts.*—Frequently the disease-producing organism is carried over winter on some part of the host, as for example, in cankers on the limbs. Again, diseased fruit or leaves dropping to the ground may harbor the fungus over winter and furnish a means of reinfection in the spring. Such “starters” should be destroyed in so far as is

practical. The labor involved in some cases is so great as to make this method impracticable.

3. *Rotation of Crops*.—This method of control is based on the fact that many fungi live in the soil or on decayed parts of the plant which cannot be conveniently removed. If the crop upon which the disease occurs is followed for a year or so by crops not subject to it, the fungus frequently dies out to a large extent. This method is not so applicable to a fruit crop as to vegetable and field crops, since the former is scarcely ever a yearly crop. Crown gall of raspberries and certain root rots can frequently be controlled in this manner.

4. *Avoiding Regions Where the Disease Prevails to a Destructive Extent*.—Orchards planted on land recently cleared frequently suffer severely from Armillaria root rot. Such locations should be avoided.

5. *Selecting or Breeding Disease-resistant Varieties*.—It is a matter of common knowledge among fruit growers that some varieties are much more prone to "take" a disease than others. Almost all parasites show this selective action; for example, Jonathan apples blight much more severely than Grimes or Ben Davis. One soon comes to the opinion that this or that variety has in it an inherent quality which makes it more or less subject to a certain disease. While this is in the main true, there are frequently striking exceptions to any general statement regarding the relative resistance of a variety.

It should not be forgotten, however, that there does exist a varietal resistance among most fruits, and the selection of varieties for commercial orcharding should be governed by this fact as well as by the relative market value of the varieties. It would, for example, be unwise to plant Smith Cider apples in a region where blotch is prevalent, or Cumberland raspberries where anthracnose is known to cause serious damage, if other equally good resistant varieties could be used.

There are few varieties which are absolutely immune to a disease. Relatively speaking, however, those varieties which are highly resistant are said to be "free" from the disease, and from a commercial standpoint they are classed as immune varieties.

Under most of the diseases described the varietal susceptibility is given for *conditions as they prevail in Illinois*, based on information received from a large number of growers, together with observations extending over a number of years by members of the horticultural staff of the Illinois Experiment Station.

6. *Destroying the Host Plants which Harbor One Stage of the Parasite*. Destruction of the red cedar to prevent the development of apple rust is an example of this method of disease control.

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APPENDIX

BACTERIOLOGICAL LITERATURE

The "literature" of a science consists of all publications dealing with it. Two general groups of publications are available. The first, consisting of text-books, etc., may be passed over without much discussion for they are based on the publications in the second group. The latter group, consisting of scientific journals, proceedings of the learned societies, monographs, etc. constitute the final basis on which knowledge in the science rests. Books, unless they report data from research investigations, are founded upon such literature. Research workers the world over are recording their data in such publications to which other investigators must go. Writers, also, have to resort to this source for information. This would be well illustrated for the introductory student if he would page through a volume of the *Journal of Bacteriology*. He would notice published papers on a great many different subjects.

A few of the more important publications in both groups are given below:

Group I: Text-books

- ABBOTT, A. C. *The Principles of Bacteriology—A Practical Manual for Students and Physicians.* Lea & Febiger, Philadelphia, 1921.
- ALLEN, P. W. *Industrial Fermentations.* The Chemical Catalogue Co., New York, 1926.
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- GILTNER, W. Laboratory Manual in General Microbiology. John Wiley & Sons, Inc., New York, 1926.
- GREAVES, J. E. Agricultural Bacteriology. Lea & Febiger, Philadelphia, 1922.
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Abstracts.—It would be difficult if not quite impossible for a scientist to follow each of the numerous publications in which articles in his field might be published. In order to assist, so-called abstract journals are published. An abstract might be defined as a short digest of a long paper. It states briefly the pertinent facts in the original paper. These abstracts are sent by those who make them to a central office where they are classified for publication. The following is an abstract taken from an abstract journal. It will illustrate the above discussion.

The dish towel as a source of tuberculous infection. C. Floyd and L. Sikorsky (Amer. Rev. Tuberculosis, 7 (1923), No. 2, pp. 117–119).—The possibility of dish towels being a source of tuberculous infection in homes where there are active cases of tuberculosis was studied by the inoculation of guinea pigs with washings of dish towels used by tuberculous patients.

In the series of twenty-five cases thus examined, no positive results were obtained. Negative results were also obtained in three control experiments in which gauze was thoroughly impregnated with tubercle bacilli and then thoroughly washed, after which tests were made as in the case of the dish towels. It is thought that the most reasonable explanation of these negative results is that any viable tubercle bacilli which might have been caught in the meshes of the dish towel were either killed or weakened by the strong alkali soap or soap powder used in washing the dishes and towels.

The original paper from which this abstract was prepared was three pages in length. The abstractor used but twelve lines for his “digest.” One recent volume of Abstracts of Bacteriology contained abstracts of 2331 different papers.

The following are useful abstract journals.

Abstracts of Bacteriology (now continued as Biological Abstracts).
Chemical Abstracts:

Biological Abstracts.
Experiment Station Record.
Bulletin de l'Institute Pasteur.

Group II, Journals. etc.

American Journal of Public Health
Annales de l'Institute Pasteur
Archiv für Hygiene.
British Medical Journal
Centralblatt für Bakteriologie
Comptes Rendus des Séances de l'Académie des Sciences
Comptes Rendus des Séances de la Société de Biologie
Journal of the American Medical Association
Journal of Bacteriology
Journal of Dairy Science
Journal of Hygiene
Journal of Infectious Diseases
Journal of Medical Research
Journal of Pathology and Bacteriology
Lancet.

GLOSSARY

- Aberrant:** Deviating from a type taken as the normal.
- Abiogenesis:** Spontaneous generation; the production of animate from inanimate matter.
- Abrasion:** A breaking of the skin or other membrane.
- Abscess:** A limited area containing pus.
- Abstract:** A short condensed report of a longer publication.
- Accessory:** Assisting; supplementing.
- Acne:** Inflammation of the sebaceous glands.
- Activator:** A substance which activates, such as co-ferment.
- Acute:** Severe, sharp.
- Acute disease:** One which reaches a crisis quickly.
- Aeration:** Admixture with air.
- Aerial:** Extending into the air; pertaining to air.
- Aerobe:** Organism requiring oxygen for life.
- Aerobiosis:** Life requiring oxygen.
- Aerogen:** A microorganism which forms gas during its metabolism.
- Agar (Agar-Agar):** A higher carbohydrate used in media.
- Agglutinate:** Clumping; a bringing together.
- Agglutination:** The act of bringing together.
- Agglutinin:** A substance formed in the blood of animals which agglutinates an antigen; an immune body, an antibody.
- Ague:** Intermittent fever such as occurs in malaria.
- Albicans:** White.
- Albuminate:** A salt formed with albumin.
- Alcoholase:** An enzyme which changes ethyl alcohol to acetic acid.
- Algae:** Microorganisms mostly aquatic cryptogams.
- Algicide:** An agent which destroys algae.
- Allergy:** The reaction shown by an organism against a substance for which it has been sensitized.
- Alveolar:** A honeycomb structure.
- Amboceptor:** A substance in blood serum which unites complement with cells.
- Amicrobic:** Free of microbes; not caused by bacteria.
- Amino acid:** Building stones of proteins; amino compounds of fatty acids.
- Amphitrichous:** Flagella at each end of the cell.
- Anabolism:** Constructive function of living cells.
- Anaerobe:** An organism which does not require air.
- Analytic:** Separation into parts; the destructive function in metabolism is analytic.
- Anaphylaxis:** Hypersusceptibility to a foreign protein.
- Anastomosis:** A union of hollow organs or cells.
- Angina:** Feeling of suffocation.
- Antagonism:** Antibiosis; opposition.
- Anthracid:** Like anthrax.

Anti-: Prefix meaning against.

Antibiosis: Antagonism; a harmful association of one or more organisms.

Antibody: An agent in the blood of animals which reacts with (destroys in some cases) other substances called antigens.

Antidote: A preparation given to counteract a poison.

Antienzyme: A substance counteracting the action of enzymes.

Antifermentative: Preventing fermentation.

Antigen: A substance which, when injected into animals, stimulates the appearance of antibodies or immune bodies.

Antiseptic: A substance which represses the development of microorganisms.

Antiserum: A preparation from the blood of animals (usually blood serum) which contains specific immune bodies.

Antitoxin: A preparation which counteracts a toxin.

Antizymotic: A substance preventing fermentation.

Arthritis: Inflammation at a joint.

Arthrospore: A spore formed from the entire cell.

Asexual: Not sexual.

Ascomycetes: A group of fungi.

Ascospore: A spore formed in an ascus.

Ascus: A sac-like structure in which ascospores are borne.

Asepsis: Absence of microorganisms or septic material.

Aspergillosis: An infection in which an aspergillus is the etiologic agent.

Assimilation: See Anabolism.

Atrichous: Without flagella.

Attenuate: To devitalize or weaken.

Autogenesis: Self-production.

Autogenous: Self-produced; of personal origin.

Autoinfection: Self-infection.

Autointoxication: Self-poisoning by poisons formed within the body.

Autolysis: Self-digestion.

Autotoxin: A toxin or poison originating within the body.

Autotrophic: Bacteria which are able to get along with carbon dioxide and inorganic salts.

Azymous: Without fermenting agents; unleavened.

Bacillicide: An agent which destroys bacilli.

Bacilliform: Having the shape of a bacillus; bacillus-like.

Bacillosis: A state of having bacilli present.

Bacillus: A genus of Schizomycetes.

Bacteremia: The presence of bacteria in the blood stream.

Bactericidal: Destroying bacteria.

Bacterin: A bacterial vaccine; suspension of dead bacterial cells in oil, saline, etc.

Bacteriolysin: An immune body which dissolves bacterial cells.

Bacteroid: Growth forms of *Rhizobium leguminosarum* (*Bacillus radicola*).

Biogenesis: Genesis of living beings from living beings.

Biolysis: Decomposition by living beings.

Bios: An accessory substance supposed to be necessary for growth of yeasts.

Blanching: A preliminary step in the canning of foods.

Blastomyces: A genus of fungi reproducing by budding.

Bovine: Having to do with cattle.

Brownian Movement: Oscillatory movement of particles; not a translocation in space.

Canker: A diseased area in the bark of trees and shrubs.

Capsule: A mucilaginous envelope surrounding certain bacterial cells.

Carrier: An individual who harbors and excretes pathogenic bacteria.

Catabolism: Breaking down of body tissue.

Catalyst: An agent which accelerates a reaction which is going on very slowly.

Cell: A bit of protoplasm with a nucleus.

Certified milk: Milk produced under a legal contract between a dairyman and a Medical Milk Commission.

Cess Pool: A contrivance for the anaerobic decomposition of the organic matter in sewage.

Chemoanalytic: The breaking down of complex compounds releasing energy.

Chemosynthetic: Synthetic reactions utilizing energy from chemical changes.

Chemotaxis: Reaction of an organism to chemicals, may be positive or negative.

Chlorination: Addition of chlorine; use of chlorine in disinfection.

Chromogenic: Forming pigments.

Chromoporous: Colorless bacteria which secrete a pigmented compound.

Chronic Disease: A disease of slow onset and progress.

Cilium: A thread-like appendage by which certain microorganisms propel themselves.

Class: A division in classification of living beings.

Classification: A systematic, reasonable arrangement of data or facts.

Coagulation: Clotting.

Coccus: A round bacterial cell.

Coenocytic: Non-septate structure of molds.

Columella: The central axis of the spore case about which the conidia are formed in molds.

Communicable Disease: A disease which may be transmitted.

Complement: A heat-labile substance found in blood.

Conidiophore: A thread or stalk bearing conidia.

Conidium: An asexual spore formed by fungi.

Conjugation: Reproduction by union of one organism with another.

Contact: Mutual touching of two agents.

Contagious Disease: A disease believed to be contracted indirectly, as by air, etc.

Contamination: Infection with septic matter.

Crenothrix: A genus of Schizomycetes.

Cryptococcus: A group of pathogenic yeast-like fungi.

Crystalloid: Having to do with crystals.

Cycle: An orderly succession of the various stages through which elements are made to pass.

Cyst: A resistant sac-like structure.

Cytology: Science of cell theory.

Cytoplasm: The protoplasm in cells.

Decay: Aerobic decomposition of protein matter.

Denitrification: Reduction of nitrogenous compounds with liberation of nitrogen.

Desensitize: To rid of susceptibility of anaphylaxis.

Dichotomy: Division into two parts; dichotomous branching.

Digestion: Breaking down of foods for passage through cell wall.

Diplococcus: Two round cells with the adjacent surfaces somewhat flattened.

Dissaccharide: A sugar composed of two molecules of monosaccharides.

Disease: An abnormal condition of body or mind.

Disinfect: To destroy infectious matter.

Dissemination: Spreading of pathogenic organisms.

Dissimilation: *See* Katabolism.

Dysentery: Inflammation of the intestinal mucosa; bloody stools.

Double Seam: The closure applied to a tin container for preserving food.

Effluent: That which flows from.

Endemic: Disease which is always present.

Endo: Prefix meaning inside.

Endospore: A spore formed in a cell.

Energy: Power to do work.

Enteric: Having to do with the intestines.

Enteritis: Inflammation of the intestines.

Epidemic: An unusual number of cases or communicable disease within a restricted area and time.

Erepsin (ereptase): An enzyme which splits peptones to amino acids.

Etiology: Science of the causes of disease.

Exhaust: The procedure in canning foods by which it is attempted to remove air from the food before the can is closed.

Extra: Prefix meaning outside.

Extracellular: Outside of the cell.

Extraneous: Foreign to the consideration.

Exudate: A substance thrown out; pus.

Facultative: Not obligatory; indifferent.

Families: A group of tribes; names of families end in *oidea*.

Fauna: The animal life of an area.

Fecundation: Fertilization.

Ferment: *See* Enzyme.

Fermentation: Decomposition of carbohydrates by living cells or enzymes.

Fertilization: Impregnation.

Filar: Filamentous; thread-like.

Fission: Reproduction by division.

Fixation: The act of holding fast.

Flagellum: A whip-like appendage on a cell.

Flat sour: A type of spoilage of certain canned foods; formation of acid without gas.

Flora: The plant (bacterial) life of a substance or area.

Focal: Having to do with a focus or point.

Fomite: An agent, usually inanimate, by which infection is spread.

Food: A substance which may be used for growth or repair by living organisms.

Fruiting Body: A complex, spore-bearing structure, usually with a definite shape.

Fungus: A plant of simple structure lacking chlorophyll. It has no root, stem or leaf and reproduces by spores, usually asexual in nature.

Fusiform: Spindle shaped.

Gangrene: Death of tissue usually accompanied by putrefaction.

Target: Inflammation of tissues in cow's udder.

Gastric: Having to do with the stomach.

- Gastritis:** Inflammation of the stomach.
- Gelose:** Agar-agar, or the gelatizing principle thereof.
- Germ:** A microorganism.
- Germ Tube:** The first shoot formed in the germination of a fungus spore.
- Germicidal:** Destructive to germs.
- Germination:** The process of germinating; the beginning of vegetation or growth in a seed or plant; the first development of germs either animal or vegetable.
- Gland:** A secretory organ.
- Globulin:** A group of proteins.
- Growth:** Increase in cell mass.
- Haptophore:** That portion of toxin molecules which bind them to body cells.
- Health:** State of well-being in body and mind.
- Heliotropism:** Reaction of living organisms to light; chemotaxis induced by light.
- Hemolysin:** A cytolytic which destroys red blood corpuscles.
- Hemolysis:** Destruction of red blood corpuscles (erythrocytes).
- Hemolytic:** The property of causing hemolysis.
- Heterotrophic:** Said of bacteria which cannot use inorganic nitrogen and carbon for growth.
- Homogeneous:** Uniform throughout.
- Homologous:** Having the same function.
- Host:** A living agent on which another lives as a parasite.
- Humoral:** Having to do with the natural body fluids.
- Hydrolysis:** Decomposition by means of water.
- Idiosyncrasy:** A peculiarity; said of those especially susceptible to certain proteins.
- Immune Bodies:** Agents in the blood of animals which are responsible for immunity.
- Immunize:** To confer immunity.
- Inactivate:** To render inactive; to destroy.
- Incubation:** That period in disease between exposure and the first symptoms; also, the procedure in the culture of microorganisms by which their growth is favored by proper temperature.
- Infect:** To communicate pathogenic bacteria.
- Infection:** Entrance and growth of a parasite in a host.
- Infectiousness:** The degree of infectiousness.
- Infectious disease:** A disease secured by infection with a living cell.
- Inflammation:** Condition of tissues in response to irritation, accompanied by heat, pain, swelling, etc.
- Infra:** below; beneath; under; after. Often used in prefix.
- Infundibuliform:** Funnel-shaped.
- Inject:** To force into.
- Inoculation:** The introduction of material such as a virus into an animal system.
- Intercalary:** Placed between.
- Intoxication:** A poisoned state of the blood.
- Intracellular:** Within the cell.
- Intramuscular:** Into the muscle.
- Intravenous:** Within a vein.
- Invertase:** Enzyme which inverts cane sugar.
- Involution:** Return to type; a retrogradation.
- Isogamy:** Sexual union of two gametes of equal size.

Isolate: To separate a microorganism from others.
Isotonic: A solution having the same density as any cell under study.

Karyokinesis: Indirect (mitotic) division of cells.
Katabolism: Breaking down of body tissue.

Lactase: The enzyme which acts upon lactose.
Laryngeal: Having to do with the larynx.
Lesion: A change in the tissues caused by injury or disease.
Lethal: Deadly, poisonous, fatal.
Leucocyte: A white blood corpuscle.
Lipase: The enzyme which splits fats.
Liquefaction: Conversion of a solid or semisolid into a liquid.
Lobate: Provided with lobes.
Lophotrichous: Tuft of flagella at one end of the cell.
Lysin: A cytolytic substance in blood serum.
Lytic: The property of dissolving cells.

Macro-: Prefix meaning large.
Macrobiosis: Long life.
Malt: Partially germinated barley, rich in amylase.
Mash: A decoction of grains or fruits.
Medical Milk Commission: A committee of physicians for the control of the production of clean milk; certified milk.
Medium: Food materials upon which microorganisms are grown.
Metabiosis: The dependence of one organism on another.
Metabolism: The processes by which food is altered by living organisms for growth and energy.
Metatrophic: Bacteria which use organic matter.
Micro: Prefix, meaning small.
Microbe: A microorganism.
Micrococcus: A genus of Schizomycetes.
Micrometer: An instrument for measuring microscopic objects.
Micromillimeter: One millionth of a meter; a micron.
Micron: One one-thousandth of a millimeter; one twenty-five thousandth of an inch.
Microscope: An instrument for viewing microscopic objects.
Mil: One cubic centimeter.
Mille: One thousand.
Mitochondria: Granules in animal cells.
Mitosis: Indirect division of the nucleus; karyokinesis.
Moniliform: Beaded.
Monosaccharide: A sugar which cannot be split into simpler sugars.
Monotrichous: One flagellum at one end of the cell.
Morphology: Science of the shape and structure.
Motility: Ability to move about.
Multiplication: Increase in number of individuals in a culture of microorganisms.
Must: Expressed juice of the grape unfermented.
Mutation: Change in property.
Mutualism: Symbiosis.
Mycelium: The cotton-like structure of threads of a fungus.
Mycology: The science of fungi.

Nitrification: Oxidation of nitrogenous compounds to nitrates.

Obligate: Necessary.

Order: A group of families. Names of orders end in *ales*.

Oospore: A sexual spore of downy mildew.

Opsonin: An antibody in blood serum which stimulates phagocytosis.

Organic: (*Different meanings.*) Said of substances derived from living organisms.

Osmosis: Passage of liquids through membranes.

Oxidase: An enzyme which brings about oxidations.

Pandemic: A world- or nation-wide epidemic.

Parasite: An organism which gets its living from another.

Pasteurization: The heating of liquids, usually milk, to temperatures below the boiling-point.

Pathogen: A disease-producing microorganism.

Pathology: The branch of medicine dealing with the structural or functional changes in the body due to disease; the science of disease.

Pellucid: Transparent, not opaque.

Pepsin (peptase): The enzyme which changes proteins into peptones.

Peptonization: The process of converting into peptones.

Perithecium: An ascus-bearing fruiting body.

Peritonitis: Inflammation of the peritoneum.

Peritrichous: Having flagella around the cell.

Phagocyte: A cell which ingests other cells.

Phagocytosis: The ingestion of invading bacteria by phagocytes.

Photosynthesis: The building of complex substances by the energy in light.

Phylum: A division of the animal kingdom.

Phytozoon: A zoophyte.

Plasma: Fluid portion of the blood.

Plasmolysis: The removal of water from a cell causing death.

Polar: At the end.

Pollution: Making impure; presence of extraneous matter.

Polymorphism: Many shapes or structures.

Polyvalent: Applied to a bacterial vaccine composed of many strains of the same organism.

Postulate: An established truth; a law.

Precipitin: An antibody which precipitates or throws down its antigen.

Preservative: An agent which represses spoilage of foods.

Process: The attempt to sterilize canned foods by heat.

Proenzyme: An incompletely formed enzyme.

Proferment: A substance from which an enzyme is formed or a substance which is necessary for its action.

Proliferation: Multiplication of cells.

Prophylaxis: Prevention of infection.

Proteolysis: The splitting or decomposition of proteins.

Proteolytic: Having the power of splitting proteins.

Protista: A group of lower plant and animal forms proposed by Haeckel.

Prototrophic: Bacteria utilizing inorganic substances.

Pseudo: Prefix meaning false.

Pseudoglobulin: One of the proteins in globulin; the other is euglobulin.

Pseudomonas: Genus of Schizomycetes with polar flagella.

Ptomaine: A nitrogenous base formed in putrefaction.

Punctate: Having numerous points.

Pus: The product of suppuration.

Pyemia: A septicemia characterized with the formation of internal abscesses.

Pyorrhea: Pus formation.

Quarantine: Period of isolation for those ill with disease or contacts.

Racemose: Like a bunch of grapes.

Ramose: Branching.

Receptor: That part of a cell which anchors materials to it.

Reproduction: The formation of offspring by living agents.

Reticular: Network construction.

Retting: The process by which linen is made from flax.

Rimous: Cracked, with fissures.

Saccharification: Change to sugar.

Sanitarian: A public health officer.

Sanitary: Promoting health; clean.

Saprophyte: An organism living on dead organic matter.

Schizogony: Reproduction by the formation of endospores.

Schizomycetes: Fission fungi.

Science: An orderly arrangement of facts; classified knowledge.

Sclerotium: The blackish mass formed by some fungi.

Scum: Surface growth.

Sedimentation: The act of settling out.

Sensitize: To render sensitive or anaphylactic.

Septate: Having cross-walls.

Septicemia: A diseased condition caused by the presence of pathogenic bacteria in the blood; so-called blood poisoning.

Septic tank: A contrivance in which the solid matter in sewage is decomposed by anaerobic bacteria.

Septum: A wall.

Serodiagnosis: Diagnosis with the aid of sera.

Serology: The science of disease diagnosis and treatment with sera.

Serum: The fluid portion of the blood, milk, etc.

Sewage: The waste matter in sewers.

Sewerage: System of pipes, etc., for carrying sewage.

Sexual: Pertaining to sex.

Sheath: A cylindrical tube surrounding organs or cells.

Soluble: Capable of being dissolved; also used to designate penetrability of enzymes through a membrane.

Species: Part of a genus.

Specific: Having a special function.

Spirillum: A genus of bacteria.

Sporangiophore: The hypha or mycelial thread which bears a sporangium.

Sporangium: A membrane containing asexual spores.

Spore: The resistant stage in the cycle of some cryptogams.

Sporulation: The act of forming spores.

Sterile: Free from living agents.

Sterilization: Destruction of all living matter.

Stratiform: Formed in a layer.

Streptococcus: A genus of cocci.

Substrate: The material upon which an enzyme or fermenting agent acts.

Suppuration: Formation of pus.

Susceptibility: A disposition to take disease.

Swell: A can of food with bulged ends.

Symbiosis: The harmonious living together of two organisms.

Therapeutic: The use of a substance in cure of disease.

Thermal: Pertaining to heat.

Thermolabile: Susceptible to heat.

Thermophile: A heat-loving organism.

Thermostabile: Heat resistant.

Toxemia: Poisoned state of the blood.

Toxin: Poison formed by bacteria and other forms of life.

Toxophore: That portion of toxin which carries the poisoning power.

Transfusion: Transfer of blood from one person to another.

Tribes: A group of genera. Names of tribes end in *ae*.

Tubercle: A nodule.

Ulcer: An open sore; suppuration on the surface.

Ultra: Beyond.

Vaccine: Lymph from cow pox vesicles; a substance used in prophylactic inoculation.

Vermiform: Worm shaped.

Vesicle: A small blister.

Virulent: Active; poisonous; infectious.

Virus: An agent which may cause disease.

Vivisection: Scientific experimentation on living beings.

Wort: Filtered mash used in fermentation or for the propagation of certain micro-organisms.

Zymogen: A substance from which an enzyme is formed.

Zoophyte: A plant-animal.

Zymotic: A germ disease; having to do with enzymes.

A TOPICAL OUTLINE
FOR
LECTURES ON MICROBIOLOGY

A TOPICAL OUTLINE FOR LECTURES ON MICROBIOLOGY

1.—General Bacteriology Lectures.

General remarks.

Historical discussion.

Spontaneous generation (biogenesis vs. abiogenesis).

Germ theory of fermentation (early work).

Germ theory of disease (early work).

Early work in agricultural bacteriology.

Development of the lens and microscope.

Present day situation. Possible future developments.

Consult: Loey, W. A., *Biology and Its Makers*.

The Text: Tanner, F. W., *Bacteriology*.

Garrison, F. H., *An Introduction to the History of Medicine*.

Libby, W., *The History of Medicine*.

2.—Systematic Relationships.

General remarks.

Grouping of different forms of life.

Haeckel's proposal.

Characteristics of plants and animals.

General principles of systematics.

Kingdoms, phyla, classes, orders, families, tribes, genera, species.

Are bacteria plants or animals?

Fungi. Characteristics.

Distribution of bacteria.

Temporary habitat; where not found.

Role of bacteria in nature's plan.

Consult: Conn, H. W., *Biology, An Introductory Study*.

Lecture Notes and Chapters II and IV in the Text: Tanner's *Bacteriology*.

3.—The Cell.

General remarks.

History of cell theories.

Constituents of cells.

Protoplasm. (Chemistry, structure, general properties, etc.).

Morphology of cells. (Nucleus, centrosome, vacuoles, etc.).

Reproduction of cells.

Structure.

Consult: Wilson, E. B., *The Cell*.

Marshall, C. E., *Microbiology*, Part I, Chapter 1 (P. Blakiston's Son & Co., Philadelphia)

Minot, C. S., *Modern Problems in Biology*.

4.—Morphology of Bacteria.

General remarks.

Shapes and sizes.

Higher bacteria, true bacteria (Eubacteria) and lower bacteria.

Filterable viruses. Bacteriophage.

Monomorphism and pleomorphism.

Mutations, involution forms, life cycles.

Methods of measuring bacteria.

Size and weight of typical bacteria.

Structure of bacteria.

Cytoplasm. (Vegetative and sporoplasm.)

Cell wall. Capsules.

Organs of locomotion. Brownian movement.

Nucleus. (Are nuclei present in bacterial cells?)

Sporulation.

Endospores; Clostridium;

Reproduction.

Consult: Marshall's Microbiology.

Lecture Notes and Chapter IV in Text: Tanner's Bacteriology.

Löhnis and Freds, Agricultural Bacteriology.

5.—Classification of Bacteria.

General remarks.

Difficulties encountered in classification of bacteria.

Early classifications.

Recent classifications.

Migula's, Lehmann and Neumann's.

Classification by committee of the Society of American Bacteriologists.

Bergey's modification.

Characters used in classifications.

Physiological and morphological.

Nomenclature of bacteria.

Descriptive chart and index number.

Common groups of bacteria.

Consult: Bergey's Manual of Determinative Bacteriology.

Lecture Notes and Chapter VI in Text: Tanner's Bacteriology.

Buchanan's General Systematic Bacteriology (Williams & Wilkins Co.).

Breed, R. S., The Present Status of Systematic Bacteriology. Jour. Bact. 15 (1928) 143-163.

6. The Molds.

General remarks.

What are molds? General structure.

Reproduction of molds.

Sexual and asexual spores.

Properties of mold spores.

Germination of spores.

Mucors.

Penicillia.

Aspergilli.

Pathogenic molds.

Aspergillosis; alternaria infections, etc.

Consult: Lecture Notes and Chapter VII in the Text: Tanner's Bacteriology.

Jørgensen, A., Microorganisms and Fermentation (C. Griffin & Co., Ltd., London).

7.—The Yeasts.

General remarks.

What are yeasts? Taxonomic situation.

Shapes of yeast cells.

Structure of cells.

Cell membrane, nucleus, vacuoles, granules.

Ascospores and their formation.

Properties of ascospores.

Habitat of yeasts.

Classification of yeasts.

Industrial yeasts.

Top, bottom, distillery.

Preservation of yeast (pressed yeast).

Therapeutic use of yeast.

Pathogenic yeasts.

Consult: Lecture Notes and Chapter VIII in the text.

Guilliermond, A., *The Yeasts* (translated by F. W. Tanner; John Wiley & Sons, Inc., New York).

Jørgensen, A., *Microorganisms and Fermentation*.

8.—Protozoa.

General remarks.

What are protozoa?

Taxonomic situation.

Relation to bacteria.

Morphology.

Cytoplasm; Nucleus; centrosome, etc.

Nutrition of protozoa.

Reproduction of protozoa.

Sporulation.

Classification.

Sarcodina; Mastigophora; Infusoria, Sporozoa.

Pathogenic protozoa.

Sleeping sickness; malaria; Texas fever.

Consult: Lecture Notes and Chapter IX in the Text: Tanner's Bacteriology.

9. Action of Physical Agents on Bacteria.

General remarks.

Light: sunlight, ultraviolet light; the spectrum.

Temperature.

Grouping of bacteria according to temperature.

Thermophilic, mesophilic, and psychrophilic.

Temperature characteristics of pure cultures.

Maximum, optimum and minimum temperatures.

Thermal death points of bacteria.

Various factors influencing thermal death points.

Relation of temperature to changes by bacteria.

Consult: Marshall's Microbiology and Lecture Notes.

Chapter X in the Text: Tanner's Bacteriology.

10.—Action of Physical Agents on Bacteria.

Destructive action of high temperatures—sterilization methods.

Dry heat.

Incineration; heating direct in flame.

Hot air oven.

Moist heat.

Boiling.

Free flowing steam. Arnold sterilizer. Low pressure.

Fractional or discontinuous method.

Continuous method.

High-pressure steam.

The student should know the precautions to be heeded in the employment of the methods mentioned above as well as the times and temperatures.

Consult: Lecture Notes and Various Reference Texts.

Chapter X in the Text: Tanner's Bacteriology.

11. Action of Physical Agents on Bacteria. III.

Moisture.

Osmosis and osmotic pressure.

Hypotonic, isotonic and hypertonic solutions.

Plasmolysis and plasmoptysis.

Pressure.

Effect on pure cultures; significance.

Attempted used in food preservation.

Agitation or mechanical shock.

Gravity.

Electricity.

Consult: Lecture Notes and the Various Reference Texts.

12.—Effect of Chemical Agents on Bacteria-Disinfection.

General remarks.

Reaction of microorganisms to chemicals.

Sterilization and disinfection—differentiation.

Disinfection and disinfectants.

Characteristics of a good disinfectant.

Factors influencing disinfection.

Mode of action of disinfectants.

The halogen compounds as disinfectants.

Chlorine compounds.

Liquid chlorine, gaseous chlorine, calcium hypochlorite,
chloramine T, dichloramine T, etc

Iodine compounds.

Free iodine, iodoform, etc.

Consult: Lecture Notes and the Various Reference Texts.

Chapter XI, in the Text: Tanner's Bacteriology.

13.—Effect of Chemical Agents on Bacteria. II.

The phenolic group of disinfectants.

Phenol or carbolic acid.

Uses: Advantages and disadvantages.

The cresols, ortho-, meta-, paracresol.

Uses: Advantages and disadvantages.

Liquor cresolis compositus, lysol, etc.

Thymol and certain allied products.

Consult: Lecture Notes and Reference Texts.

Dakin, H. D., and Dunham, E. K., A. Handbook of Antiseptics (The MacMillan Co.).

14.—Effect of Chemical Agents on Bacteria. III.

The salts of the heavy metals.

Action of such salts as disinfectants.

Mercury salts: Mercuric, chloride, mercuric iodide, etc.

Advantages and disadvantages; limitations.

Zinc salts: Zinc chloride.

Silver salts: Silver nitrate, argyrol, argonin, argentose, etc.

Copper salts: Copper sulfate, copper chloride, etc.

As disinfectants and algicides.

Miscellaneous compounds used as disinfectants.

Hydrogen peroxide, boric acid, ethyl alcohol, surgical soaps, ointments, etc.

Standardization of disinfectants.

Terminal disinfection—fumigation.

Consult: Lecture Notes and Reference Texts.

15.—Mutual Relationships.

General remarks.

General significance in nature.

Symbiosis; types and examples:

Antibiosis; types and examples.

Parasitism and pathogenesis.

Metabiosis; examples.

Commensalism.

Consult: Lecture Notes and Various Reference Texts.

Chapter XII in the Text: Tanner's Bacteriology.

16.—Nutrition of Bacteria.

General remarks.

Amount of food required. What is food?

Organic and mineral foods.

Foods for growth and building purposes.

Metabolism, katabolism, and anabolism.

Plant and animal metabolism.

Bacterial metabolism.

Foods for energy.

Respiration; aerobic and anaerobic.

Fermentation.

Autotrophic, metatrophic and heterotrophic bacteria.

Cycles of the elements.

Consult: Lecture Notes and Various Reference Texts.

Palladin-Livingston's Plant Physiology.

Chapter XIII in the Text: Tanner's Bacteriology.

17.—Growth of Bacteria.

What is growth?

Methods used by bacteriologists for measuring growth.

Growth histories of cultures.

Growth histories of single cells.

Rate of growth.

Factors influencing growth.

Consult: Lecture Notes and Reference Texts.

Chapter XIV in the Text: Tanner's Bacteriology.

18.—Enzymes of Bacteria.

General remarks.

Proof of existence of enzymes.

Nomenclature of enzymes.

Chemical changes brought about by enzymes.

Intracellular and extracellular enzymes.

Characteristics of enzymes and enzyme reactions.

Systematic arrangements of classification of enzymes.

Consult: Lecture Notes and Chapter XV in the Text: Tanner's Bacteriology.

Thatcher, R. W., The Chemistry of Plant Life (McGraw Hill Book Co., New York).

Marshall's Microbiology.

Wakeman, S. A., and Davison, W. C., Enzymes (Williams & Wilkins Co., Baltimore).

Fuhrmann, E., Vorlesungen über (Bakterienzyme, Gustav Fischer, Jena).

Buchanan, R. E., The Classification of the Enzymes of Microorganisms (Proc. Iowa Acad. Sci., 31 (1924) 107-109).

19.—Enzymes of Bacteria: Special Work.

Prepare a table with the following headings (starting from the left hand side of the page): Name of Enzyme; Substrate Acted Upon; Type of Reaction; Products.

Fill in this table with the proper information for the following enzymes: *cellulase*, *pectase*, *maltase*, *lactase*, *emulsin*, *amylase*, *invertase*, *peptase*, *tryptase*, *ereptase*, *rennin*, *alcoholase*, *lactacidase*, *endotryptase*, autolytic enzymes, reductase for litmus, methylene blue and nitrates.

The data for this work should be secured from any source of information on enzymes. The references given on the previous card will be found to be especially useful. Please have this outline ready to hand in at the quiz section at which enzymes are scheduled for discussion.

20.—Nitrogen Metabolism and Cycle.

Occurrence of nitrogen in nature.

Significance of nitrogen for living organisms.

The nitrogen cycle.

Fixation of atmospheric nitrogen

Chemical methods

Biological methods

Symbiotic methods

Non-symbiotic methods

Soil inoculation methods

Are there different species of legume bacteria?

How is nitrogen given to the plant?

Nitrification, denitrification, ammonification

Decomposition of proteins: Putrification, decay.

Consult: Lecture Notes and Pertinent Chapters in Text.

Marshall's Microbiology.

Chapter XVI in the Text: Tanner's Bacteriology.

21.—Sulfur Metabolism and Cycle.

General remarks.

Production of hydrogen sulfide.

Formation from proteins.

Formation from sulfates and sulfites.

Oxidation of hydrogen sulfide.

Oxidation of sulfur.

Sulfur bacteria.

(Other cycles—mentioned briefly without discussion.)

Consult: Lecture Notes and the Text. Chapter XVI in the Text: Tanner's Bacteriology.

22.—Water Bacteriology and Hygiene.

General remarks.

Definitions and types of water.

Relation of water to disease.

Longevity of disease bacteria in water.

Evidences of pollution; sanitary inspection.

Chemical methods of water analysis.

Bacteriological methods of water analysis.

Escherichia coli as an indicator of pollution.

Separation of *Escherichia coli* and *Aerobacter aerogenes*.

Methods of purification of public water supplies.

Private and rural water supplies.

How to have water analyzed.

Bottled and mineral waters.

Bacteriology of ice.

Consult: Lecture Notes and Chapter XX in the Text: Tanner's Bacteriology.

Prescott, S. C., and Winslow, C.-E. A., Elements of Water Bacteriology (John Wiley & Sons, Inc., New York).

23.—Bacteriology of Sewage.

Bacteriology in sewage.

General principles of sewage treatment.

Preliminary methods.

Septic tank; cess pool.

Finishing processes.

Dilution; stream pollution and improvement of streams.

Sewage farming: Relation to spread of disease.

Filtration.

Aeration: Activated sludge.

Rural or private sewage treatment plants.

Consult: Lecture Notes and Reference Texts.

Chapter XXI in the Text: Tanner's Bacteriology.

Kinnicutt, L. P., Winslow, C.-E. A., and Pratt, R. W., Sewage Disposal (John Wiley & Sons, New York).

Rosenau, M. J., Preventive Medicine and Hygiene (D. Appleton & Co., New York.)

24.—Bacteriology of Milk and Dairy Products.

Bacteria in milk; bacteria in milk in the udder.

Various factors influencing bacteria in milk.

Methods available for improving quality of milk.

Sanitary inspection.

Medical milk commissions; certified milk.

Pasteurization of milk.

Methods for determining "quality" of milk.

Laboratory procedures.

Grading of milk: Examples.

Bacteriology of butter, cheese and ice cream.

Fermented milks.

Origin: Therapeutic use of.

Consult: Lecture Notes and Chapter XXII in the Text: Tanner's Bacteriology.

References: Rogers, L. A., Fermented Milks (Bull. 319, U. S. Dept. Agr., Washington).
Rosenau, M. J., Preventive Medicine and Hygiene.

25.—Industrial Fermentations.

General remarks.

Vinegar fermentation.

Raw materials; chemistry and bacteriology of;

Various methods of making vinegar.

Textile fibers.

Flax and linen; hemp;

Deterioration of textile fibers by microorganisms.

Beverage materials.

Tea; coffee; cocoa.

Fermentation of bread, etc.

Leavening agents

Chemical vs. biological leavening agents.

Industrial ethyl alcohol.

Acetone, butanol, methanol,

Glycerol.

Lactic acid.

Carbon dioxide.

Consult: Chapter XXIII in the Text: Tanner's Bacteriology.

26.—Food Preservation.

General remarks.

Methods used in preservation of food.

Asepsis;

Low temperatures.

Freezing; cold storage; refrigerators.

High temperatures.

Boiling and cooking; pasteurization; canning.

Chemicals as Preservatives of Foods.

Sodium benzoate; salt; sugar; spices; smoking.

Chemicals formed in fermentation.

Drying (dehydration, dessication).

Consult: Lecture Notes and Chapter XXIV in the Text: Chapter XXIV.

Powell, O., Successful Canning and Preserving.

Tanner, F. W., Bacteriology and Mycology of Foods (John Wiley & Sons, Inc., New York).

27. Illness Caused by Food.

General remarks.

Several types of illness caused by foods.

Allergic reactions of foods (idiosyncrasies).

Foods which are naturally poisonous.

Poisonous varieties of mushrooms; other plants.

Poisonous fish, etc.

Foods undesirable on account of decomposition.

Bacteriology and chemistry of such decomposition.

Ptomaines and ptomaine poisoning.

Storage of foods in the opened tin can.

Foods containing metallic salts.

Typical food poisoning (botulism).

Brief historical review.

Symptoms; *Clostridium botulinum*.

Characteristics of *Clostridium botulinum*.

Food-borne infections.

Trichinosis; bacterial infections.

Consult: Lecture Notes and Chapter XXV in the Text: Tanner's Bacteriology.

Savage, W. G., Food Poisoning and Food Infections (Cambridge Univ. Press).

28. Relation of Microorganisms to Disease.

General remarks.

Discussion of different conceptions of disease.

Some theories for explaining disease.

The demonic theory.

The humoral theory (theory of the four humors).

The zymotic theory.

The zymotoxic theory.

Types of diseases.

Contagious, infectious, and communicable diseases.

Diseases of known and unknown etiology.

Koch's postulates.

Classification or tabulation of diseases.

Consult: Lecture Notes and Chapter XXVI in the Text: Tanner's Bacteriology.

Sedgwick, W. T., Principles of Sanitary Science and the Public Health (The Macmillan Co., New York).

29—Infection: General.

Pure and single infections.

Primary and secondary infections.

Mixed infections.

Modes of bacterial action.

Conditions influencing pathogenicity.

Tissue changes.

Definitions:

Infection, etiology, disease, health, susceptibility, immunity, inflammation, suppuration, lesion, toxemia, bacteremia, septicemia, pyemia, intoxication, virus, epidemic disease, endemic disease, pandemic disease; acute and chronic disease.

Consult: Lecture Notes and Reference Texts.

Gould's Medical Dictionary (P. Blakiston's Son & Co., Philadelphia, 1928).

Dorland's The American Illustrated Medical Dictionary (W. B. Saunders Co., Philadelphia, 1921).

See also Glossary at end of the Text: Tanner's Bacteriology.

30.—Transmission of Infecting Agents. I.

Longevity of disease-producing bacteria away from their hosts.

Infection by carriers.

Definitions; diseases spread by carriers.

General discussion of carrier problem.

The missed case.

Definition; menace of missed case in public health work.

Infection by fomites.

Definition; books; money; dishes and tableware, etc.

Bacteriology of dishwashing; soaps and washing powders.

Other fomites.

Air-borne infections.

General discussion; fallacies of as a method of disease dissemination.

Consult: Lecture Notes and Chapter XXVII in the Text:

31.—Transmission of Infecting Agents. II.

Contact infections.

Definition: Direct and indirect contact.

Examples of contact infection.

Insect-borne diseases.

Examples: Yellow fever, malaria.

Historical setting.

Animal-borne diseases.

Diseases transmissible from animals to man.

Tuberculosis, glanders, anthrax, etc.

Food-borne diseases or infections.

General remarks.

Discussed in another place.

Consult: Lecture Notes and Reference Texts.

Rosenau, H. J., Preventive Medicine and Hygiene.

32.—Methods of Preventing Spread of Disease.

General remarks.

Two general groups of methods: Subjective and objective.

Objective methods: Disinfection, sterilization of water, milk, etc.

Subjective methods: Preventive inoculation.

Quarantine methods.

International quarantine (maritime quarantine) significance; diseases quarantined against; quarantine stations, etc.

Federal or state quarantine.

Limitations of; examples.

State quarantine.

Enforced by state laws.

Municipal quarantine.

City ordinances.

Detention camp.

Sanitary cordon.

Isolation of patient.

References: Rosenau, M. J., Preventive Medicine and Hygiene.

Lecture Notes and Reference Texts.

Chapter XXVII in the Text: Tanner's Bacteriology.

33.—Factors Influencing Infection.

I. Remote factors.

- A. Heredity.
- B. Age.
- C. Mental state.
- D. Occupation.
- E. Housing conditions.

II. Immediate factors.

- A. The subject of infection (patient or host).
 - 1. Resistance to disease.
 - 2. External defences.
 - a. Mouth.
 - b. Stomach.
 - c. Mucous membranes.
 - d. Lungs.
 - e. Skin.
- B. The infecting agent.
 - a. Virulence or infectiosity.
 - b. Number of cells (size of the dose).
 - c. Avenue of infection.

Consult: Lecture Notes and Chapter XXVIII in the Text: Tanner's Bacteriology.

34.—Toxin Formation.

General remarks. . Intoxication.

Formation of toxins by living organisms; by animals; by plants; microorganisms.

Theories for explaining toxin formation.

Extracellular and intracellular toxins.

Characteristics of toxins and toxin reactions.

Toxins and enzymes compared.

Toxins vs. ptomaines.

Consult: Lecture Notes and Texts.

Encyclopedia Britannica.

Chapter XXIX in the Text: Tanner's Bacteriology.

35.—Protective Substances (Immune Bodies) I.

General remarks.

Antigens and antibodies.

Discussion of terms.

Serum, antitoxic serum, antibacterial serum.

Tabulation of immune bodies to be discussed.

Antitoxins

Agglutinins

Precipitins

Opsonins

Lysins.

Consult: Lecture Notes and Reference Texts.

Broadhurst, J., How We Resist Disease (J. B. Lippincott Co., Philadelphia).

Chapter XXX in the Text: Tanner's Bacteriology.

36.—Antitoxins.

General remarks on intoxication.

Production of antitoxins (antitoxic sera).

Production of toxin; animals used.

Immunizing the horse.

Collecting the blood.

Measuring strength of antitoxins in serum.

Purified concentrated antitoxic serum.

Tests for purity.

Use of antitoxins (antitoxic sera).

Allergic reactions following administration.

Size of the dose.

Reasons for failure.

The Schick test in diphtheria.

Toxin—Antitoxin administration.

The Dick Test in scarlet fever.

Different kinds of antitoxin (antitoxic sera).

Consult: Lecture Notes and Texts.

Chapter XXX in the Text: Tanner's Bacteriology.

37.—Agglutinins and Precipitins.

General Remarks on agglutinins.

Preparation of agglutinins (agglutinating sera).

Demonstration of agglutinins.

Practical applications of the agglutinins.

The Widal reaction in typhoid fever; other agglutinating reactions.

Identification of unknown bacteria.

Agglutination reactions in blood transfusion.

General remarks on precipitins.

Preparation of precipitins (precipitating sera).

Practical applications of precipitins.

Identification of blood from different species.

Use in identification of human blood.

Detection of foreign proteins in foods.

Separation of species. Use in discussions of evolution.

Consult: Lecture Notes and Texts.

Kolmer, J. A., A Practical Text-Book of Infection, Immunity, and Biologic Therapy
(W. B. Saunders & Co.), Philadelphia.

Chapter XXX in the Text: Tanner's Bacteriology.

38.—Opsonins and Lysins.

General remarks on opsonins.

Normal and specific opsonins.

Opsonic index: Significance in immunity work.

Outline of steps in method for determining opsonic index.

General remarks on lysins.

Different types of lysins.

Cytolysins, hemolysins, bacteriolysins, etc.

Significance in disease and immunity.

Consult: Lecture Notes and Reference Texts.

Jordan, E. O., General Bacteriology (W. B. Saunders & Co., Philadelphia).

Chapter XXX in the Text: Tanner's Bacteriology.

39.—Methods and Varieties of Artificial Immunity—Active Immunity.

1. Natural immunity.
2. Artificial immunity (acquired).
 - A. Active immunity—i.e. produced in an animal by an injection, by a series of injections, of non-lethal doses of an organism of its toxins.
 1. Attack of the disease.
 2. By injection of living organisms.
 - a. Attenuated in different ways.
 1. Growing in presence of oxygen.
 2. Animal passage.
 3. Abnormal temperatures.
 4. Weak antiseptics.
 5. Drying.
 - b. Virulent—non-lethal doses.
 3. By injection of dead organisms (bacterins).
 4. By injection of dead organisms sensitized by an anti-serum.
 5. By injection of filtered bacterial cultures, i.e., toxins; or of substances derived from such filtrates.

Consult: Chapter XXXII in the Text: Tanner's Bacteriology.

40.—Methods and Varieties of Artificial Immunity (Cont.) Passive Immunity.

- B. Passive immunity—i.e., produced in one animal by the injection of the serum of another animal immunized by the methods of A.
 1. By antitoxic serum, i.e., the serum of an animal highly immunized against a particular toxin.
 2. By antibacterial serum, i.e., the serum of an animal highly immunized against a particular bacterium in the living and virulent condition.

Adapted from Muir and Ritchie's Manual of Bacteriology.

41.—Theories of Immunity.

- General discussion of the whole field.
- Exhaustion theory.
- Noxious retention theory.
- Metschnikoff's theory (theory of phagocytosis).
- Ehrlich's theory (side chain theory).
- Vaughan's theory.

Consult: Lecture Notes and Texts.

Chapter XXXI in the Text: Tanner's Bacteriology.

42.—Methods of Active Immunity. I.

- Active and passive immunity defined and discussed.
- Methods of active immunity.
 - Attack of the Disease.
 - Limitations and misinformation on the subject.
 - Injection of living cells attenuated in some manner.
 - Attenuation defined and discussed.

Attenuation by animal passage.

Immunity methods in smallpox.

History of smallpox immunization.

Preparation of smallpox vaccine.

Friedmann's cure for tuberculosis.

Attenuation by abnormal temperatures.

Pasteur's anthrax vaccine. Public demonstration by Pasteur.

Attenuation by drying.

Pasteur's treatment for rabies (hydrophobia).

Injection of non-lethal doses of living organisms.

Danger and Limitations as a method in immunity.

Consult: Lecture Notes and Reference Texts.

Various Texts in Medical Bacteriology.

Chapter XXXII in the Text: Tanner's Bacteriology.

43.—Methods of Active Immunity. II.

Injection of dead microorganisms.

Discussion of the terms vaccines and bacterins.

Methods followed in preparation of bacterins.

Stock and autogenous bacterins.

Single and mixed bacterins.

Saline and lipo-bacterins.

How long does immunity last after vaccination?

Reasons for failures in preventive inoculation.

Injection of bacterial extracts.

Historical work on tuberculin.

Preparation and uses of tuberculin.

Passive immunity methods.

Have been discussed before.

Recapitulate; general discussion.

Consult: Lecture Notes and Reference Texts.

44.—Plant Diseases.

General remarks.

Economic importance of plant diseases.

Brief historical survey.

Blights:

Pear blight; blade blight of oats; blight of beans; other blights.

Leaf spots, etc.

Of celery; tomato; delphinium.

Rots:

Cabbage rot; cauliflower rot; carrot rot; other rots.

Wilts:

Sweet corn wilt; other wilts.

Galls:

Crown gall.

Analogy between crown gall and cancer.

Control of plant diseases.

Consult: Chapter XXXIII in the Text: Tanner's Bacteriology.

Anderson, H. W., Diseases of Illinois Fruits (Illinois Agr. Exp. Sta., Circular 241, 1920)

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